LOW COST AUTOMATED BLOOD CULTURE SYSTEM

by

Tal Gottesman

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ABSTRACT

Sepsis is a systemic medical condition causing serious risk to health and life. This condition is most often diagnosed by culturing a specimen of patient blood or body fluid for bacteria. The health of patient populations in emerging economies would be aided by better diagnosis and treatment of sepsis. Unfortunately factors such as cost, ease of use, unreliable power and environmental control in laboratory facilities make automated blood culture untenable in these locations thereby limiting diagnostic options for clinicians. A comprehensive system design is proposed which addresses an unfulfilled need for an inexpensive method and apparatus which would facilitate automated blood culture. The design describes a disposable culture tube comprising a chemical sensor and bacterial growth medium. It also describes an automated instrument which will incubate, agitate, and optically interrogate culture tubes for evidence of bacterial growth. Culture tubes which demonstrate evidence of bacterial growth are reported as positive, facilitating the diagnosis of sepsis.

Directed By: Jeff Houser

Johns Hopkins University

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1 Background:

Sepsis, (blood poisoning) is a serious medical condition caused by an immune response to infection. It is also referred to as Systemic Inflammatory Response Syndrome (SIRS) and in severe cases it leads to septic shock (sepsis –induced hypotension), organ failure and eventually death. Sepsis occurs in 1%-2% of all hospitalizations in the U.S. and some studies report that it affects 750,000 Americans per year (1, 4). It is estimated that between 28 and 50 percent of those infections result in death (2). Sepsis results in more deaths in the United States than prostate cancer, breast cancer and AIDS combined (3). The economic cost of sepsis has been estimated at $17 billion per year. Bacterial infections (bacteremia) are the most common cause of sepsis (4) and are diagnosed by culturing the blood or other bodily fluid for bacteria.

Bacterial culture involves the collection, inoculation, incubation, growth, and detection of viable organisms inside a sealed vessel. It has several market applications including blood culture, urine culture, product sterility and food safety testing. Manual bacterial culturing methods have been used in the medical field since the early 20th century to diagnose patients presenting with a fever of unknown origin. In the 20th century, semi-automated and then automated methods and equipment have been developed. These commercially available culture systems provide good performance, but are prohibitively expensive for

use in developing nations or by smaller clinics like those commonly found in rural areas.

Commercially available systems tend to incorporate intricate agitation systems and complex measurement systems which include optical filters and highly sensitive sensors. In some cases these systems also require the use a disposable culture vial which necessitates a multi-step manufacturing process and a complex sensor design which increases the cost of operation. Some examples of commercially available systems are listed in Appendix A along with a brief description of how they work and an assessment of various tradeoffs.

For the purposes of this project I designed and developed an inexpensive automated culture system which could rival or surpass the performance of predicate systems at only a fraction of the cost. The design criteria are low cost, detection accuracy and time to detection of bacterial cultures.

The specific designs of the automated instrumentation and disposable culture vial which can achieve these cost and performance targets are detailed within this document.

2 System:

2.1 Overview:

This low cost automated culture system comprises five functional parts; a specimen vial containing a growth indicator and growth medium, an incubation

system, an agitation system to enhance growth, an optical detection and signal processing system, and a computerized system of logging data and interpreting results.

2.2 Growth Indicator:

2.2.1 Theory of Operation

The growth indicator is a chemical sensor which consists of a liquid pH indicator that is separated from the growth medium by a gas permeable membrane. Figure 1 shows one possible embodiment of such a sensor. As bacteria grow, they produce carbon dioxide through respiration which migrates across the membrane and causes a change the pH of the liquid indicator. This in turn results in a color change of the indicator, which is interrogated optically and recorded. Blood also creates carbon dioxide by respiration, but is produced only in bone marrow and cannot reproduce on its own. Because the bacteria are reproducing and the blood cells are dying, the rate of change of the pH indicator will differ between a sample containing blood and a sample containing blood contaminated with bacteria.

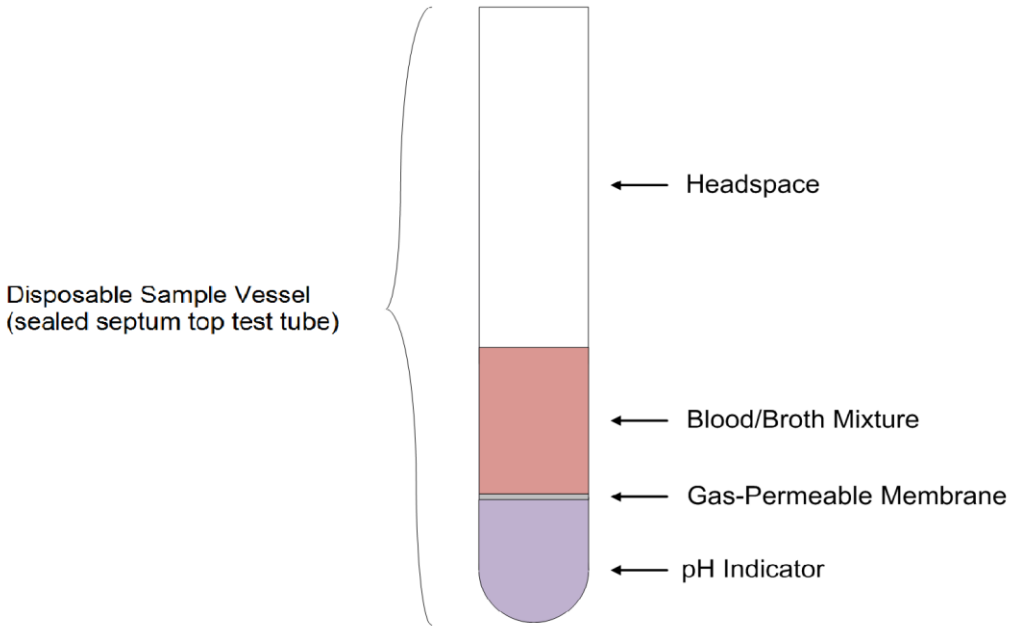


Figure 1: Chemical Sensor

2.2.2 Design

The chemical indicator selected for this purpose is Bromocresol Purple. Bromocresol Purple was selected because it is low cost, non toxic, readily available from chemical supply houses, changes color very close to neutral ph and changes between colors that are of significantly different wavelengths. Specifically, it changes from purple at a pH of 6.8 to yellow at a pH of 5.2. The color is significant because it allows for an optical detection system with a low cost design with a very large dynamic range.

A cost optimized design is realized by minimizing; the complexity of the sensor, the number of manufacturing steps involved in producing it, and the precision of the tolerances required in the manufacturing process. This is accomplished by utilizing a simple process in which the liquid pH indicator is homogenized within a

silicone matrix, creating small droplets, or micelles of indicator. The silicone sensor suspension is dispensed directly into the sample tube and allowed to cure in place. Silicone, being gas permeable, provides the necessary physical barrier between the chemical sensor and the sample. Because the indicator is uniformly distributed throughout the silicone, this design allows for the interrogation of the entire sensor cross section. This has the added benefit of making precise optical alignment unnecessary, which reduces manufacturing costs and increases reliability. Figure 2 shows the cost optimized design for the disposable specimen vial and chemical indicator that was developed for this project.

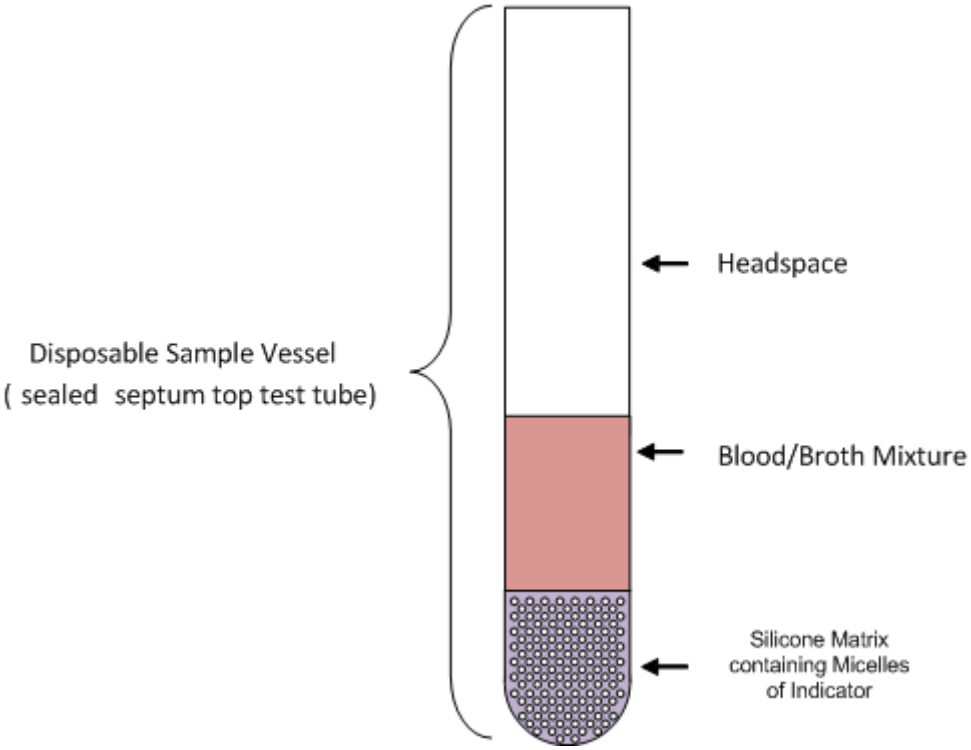


Figure 2: Cost Optimized Design for Chemical Sensor

2.2.3 Optimization

The silicone selected for the chemical sensor is Platsil 73-15. Platsil 73-15 is a 2 part platinum cure silicone commonly used in the mold making industry for theater props. Prior to curing, the liquid silicone is slightly acidic which would cause the indicator to become prematurely exposed during the curing process. This issue is overcome by pre-biasing the pH indicator solution with a base (sodium bicarbonate) to a higher pH so that the resulting mixture is pH neutral. The final pH of the sensor will therefore depend on the titrated pH of the indicator solution and on the ratio of indicator solution to silicone. The indicator solution to silicon ratio dictates the concentration of micelles and therefore affects the color density and gas permeability of the cured sensor. For these reasons it was necessary to empirically determine the optimal ratio and titration levels. Table 1 and Figure 3 show the data of the relative intensity of light transmitted through sensors created with various titration levels and sensor concentrations. The sensors were sampled using the ADC (analog to digital converter) within the microcontroller used in this design and the units are counts. The titration level and sensor concentration used in the formulation of batch number 2 provided the highest dynamic range and was therefore selected for use in this design.

Batch Number	1	2	3	4
Indicator Solution (mL)	1	2	3	4
Titration Solution (drops)	2	3	4	5
Unexposed ADC Reading	761	212	463	146
Exposed ADC Reading	1023	790	819	200

Table 1: Empirically tested Concentration Ratios

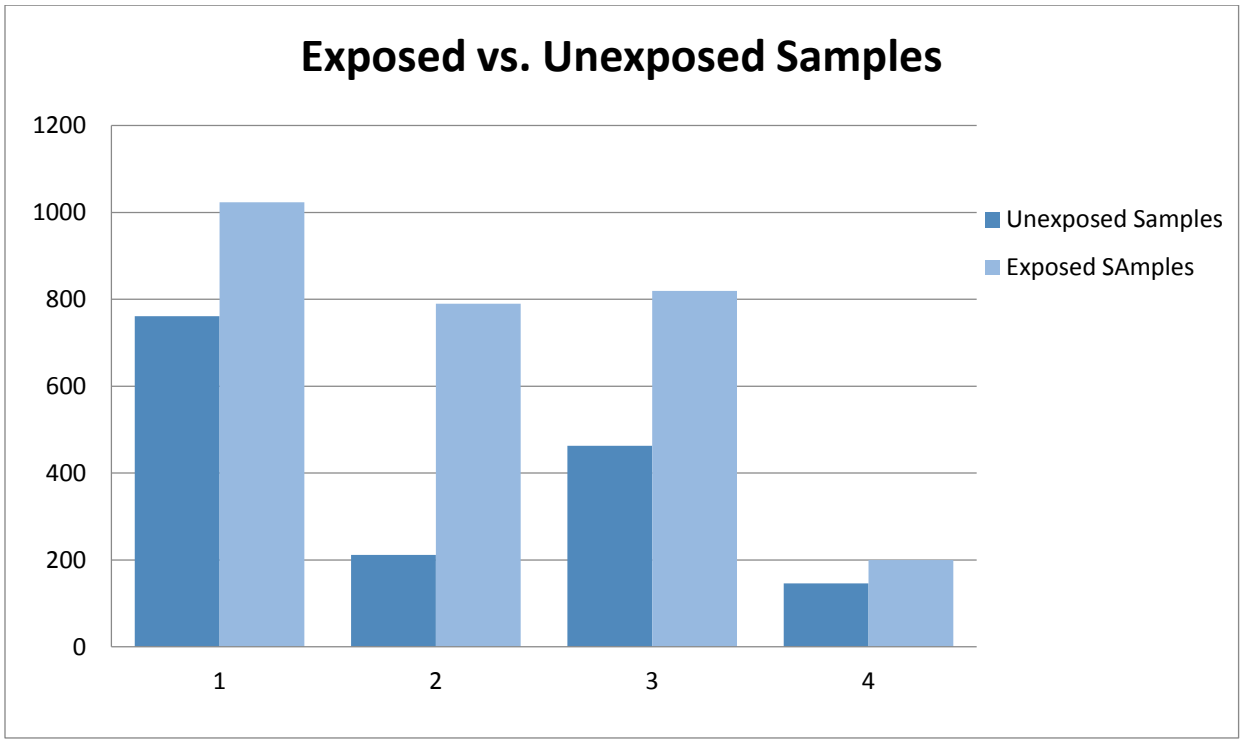


Figure 3: Dynamic Range of Sensors with Various Concentrations of Indicator

For the development of a working model of the sensor, Bromcresol Purple was dissolved in water until the water was saturated. The solution was decanted into part A of a two-part platinum-cure silicone. The mixture was vigorously whipped using a vortex mixer until the solution was broken down into small droplets (micelles) suspended in the silicone. Part B of the platinum silicone was added, and then thoroughly mixed. The entire mixture was then dispensed into test tubes and allowed to cure. Once cured, a column of sterile water was added over the top of the silicone sensor in both tubes.One sensor tube was maintained as a

control allowing only atmospheric levels of CO2 to be present (approximately .039%). The tube was then capped with a screw top cap. A second sensor tube was bubbled with 100% CO2 for 1 minute, and the tube was then capped. The tubes were the left for 1 hour to allow the migration of CO2 into the sensor. Figures 4 and 5 show the unexposed and exposed samples respectively.

Figure 4: Unexposed Sensor

Figure 5: Exposed Sensor

2.3 Optical Detection System:

The optical detection system consists of a narrow spectrum light source and a detector. For the light source, a 5mm amber LED was selected (part number C503B-AAS-CY0B0251 manufactured by Cree Inc.). This LED was designed and mass produced for outdoor sign and signal applications and therefore offers high temperature and high moisture resistance performance at a very low cost. It emits light at a peak wavelength of 591nm with a typical luminous intensity of 13000mcd at 20mA. The proposed chemical indicator is designed to provide maximum optical absorption of the source wavelength in the purple (unexposed) state, and maximum optical transmission in the yellow (exposed) state. As such, the choice of this amber LED in conjunction with the proposed chemical sensor provides a positive-going signal with increasing CO2 production. This optical system achieves excellent dynamic range without the need for expensive optical filters or sensor calibration which would add cost to the system.

An avalanche photodiode would be an ideal sensor to measure the amplitude of the transmitted light, but would cost anywhere from several hundred to several thousand dollars in single quantities, depending on the specific part. The lowest cost avalanche photodiode listed by the electronics distributor Digikey, for example, is the SD197-70-74-591 made by Advanced Photonix and has a manufacturer suggested retail price (MSRP) of $942.50 in single quantities as of November 04, 2013. This system is designed to test 12 samples at a time and therefore would require 12 avalanche photodiodes which would increase the cost significantly. Because of the prevalence of fiber optic communication networks, PIN photodiodes on the other hand, have become ubiquitous and extremely cost

effective. The PIN photodiode selected for this sensor is the PD333-SC/H0/L2 and costs $0.50 in single quantities at retail. This photodiode has a spectral bandwidth between 400nm and 1100nm with peak sensitivity at 940nm.

Ideally, the wavelength of peak sensitivity for the photodiode would match the LED dominant wavelength of 591nm, but he PD333-SC/H0/L2 has a sufficiently broad spectral response to provide good sensitivity in the area of interest (591nm). Because the system design can tolerate the slightly reduced sensitivity of the detector at this wavelength, the low cost justifies the performance tradeoff. Modulating the light source and band pass filtering the received signal would provide good immunity from ambient light. This would however add complexity and therefore cost to the system, so instead this system is designed to prevent stray ambient light from entering the optical path and reducing the signal to noise ratio. This is accomplished by operating the detection system inside of a light-tight enclosure and protecting the sensor from ambient light with the geometry of the detector design. The LED and the detector must be aligned co-axially, and recessed into wells in the sample holding block. This will effectively collimate the source light and block light from other sources (see Figure 6).

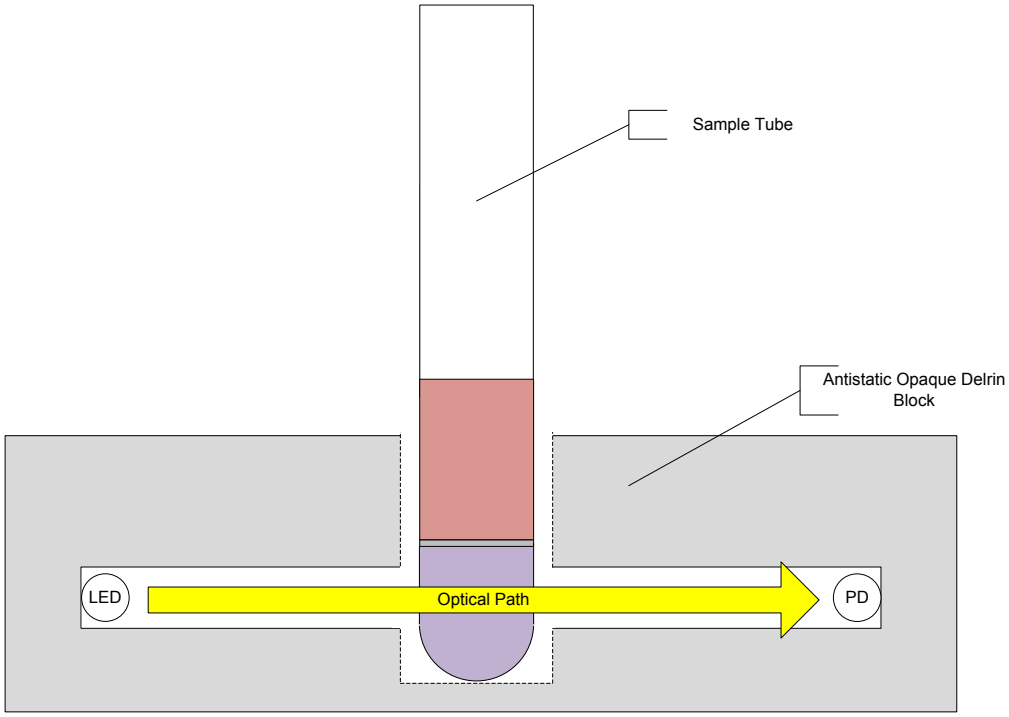


Figure 6: Optical Sensor Block Diagram

Design consideration was given to the inclusion of an optical diffuser in the measurement path. Optical diffusers scatter light across the intended optical path providing some degree of "averaging" of the path in total. This would desensitize the system to minor variations in optical alignment of the measurement system components, and would be necessary if the chemical sensor was not uniform. It was determined that a more cost effective means of providing optical diffusing without the need for an optical filter, would be to design the chemical sensor such that it acts as a diffuser as well as a sensor. The sensor design includes color-neutral optical scattering centers to eliminate the need for a diffuser. This was achieved by careful selection of a diffuse rather than clear 2 part silicone compound.

All of these design features acting in concert eliminate the need for any optical filters in the measurement system, which reduce the part count and thereby provide a significant cost benefit to the measurement system.

2.4 Signal Generation and Processing System

The signal processing path consists of an LED source and controller, an amplifier and filter circuit and an Analog to Digital Converter (ADC). All three sections are actively controlled by a single low cost microcontroller.

2.4.1 LED Control

Each of the 12 sampling stations is illuminated by a single amber LED. The LEDs are driven with a programmable constant current source under firmware control. This allows for the intensities to be varied with 128 steps of resolution in order to compensate for variations between sampling stations in optical alignment, LED forward voltage drop and amplifier stage gain. A single low cost LED driver chip (Texas Instruments TLC5929) is used drive all 12 LEDs which can be switched individually under microcontroller control to prevent light contamination between adjacent stations. The block diagram in Figure 7 shows an overview of the LED driver circuit.

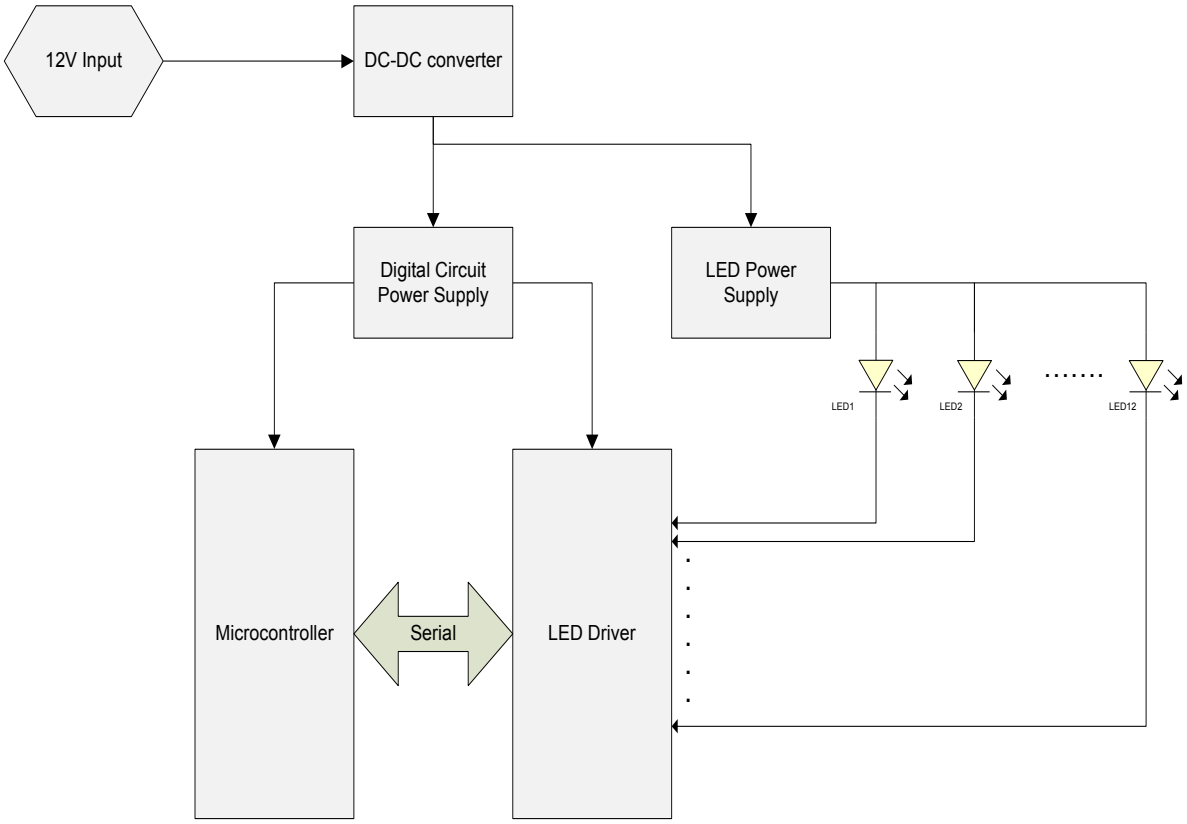


Figure 7: LED Driver Circuit

The LED driver regulates the current by means of an internal switching power supply driven at approximately 20 MHz. The power supply used to drive the LEDs is separate from the supply used to drive the amplifier and ADC to reduce the effect that the switching noise may have on the analog measurements. Both supplies are based on low cost LDOs (low dropout regulators) and are heavily filtered to minimize noise.

2.4.2 Amplifier and Filter Circuit

The first stage of the amplifier and filter circuit is a transimpedance amplifier configured in photovoltaic mode as shown in Fig 8 below.

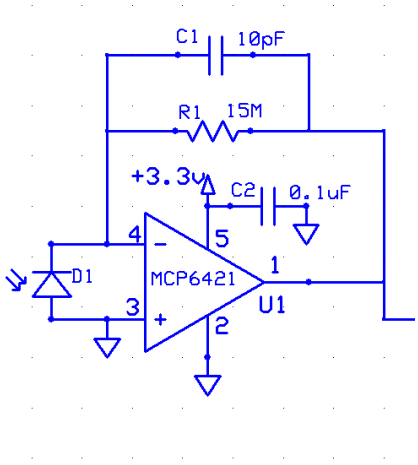


Figure 8: Transimpedance Amplifier Stage

Photovoltaic mode was chosen over photoconductive mode because response time is not critical in this application and it has the advantage of having zero dark current. The most important criteria for selection of the op-amp are; low input bias current, low input offset voltage, low input offset voltage drift, rail-to-rail input and output, single supply 3.3V operation and cost. Since the signal frequency will be approximately DC, the gain bandwidth product (GBW) does not need to be so high that it eliminates most low cost op-amps as possible candidates for this design. The Microchip MCP6421 is commercially available, low cost and meets or exceeds all of the stated requirements. This amplifier circuit will also form a low pass filter with a cutoff frequency of ~14Hz. This serves to offer

protection from the 50Hz or 60Hz noise associated with the electric grid, depending on the location.

The total gain required is a function of the mechanical alignment as well as the specific makeup of the sensor material, and has been determined empirically. To obtain the highest possible signal to noise ratio all the gain would ideally be achieved in this stage, but this is not be possible due practical limitations. Specifically, a gain limit is imposed by surface leakage currents on the printed circuit board (PCB), which reduces the effective impedance in the feedback path of the circuit. This has been minimized by the use of guard rings in the layout to shunt stray currents to ground and with a conformal coating on the amplifier stage to keep moisture out of the components and PCB. Minimal additional gain is achieved in the second (filter) stage.

The second stage is a second order active low pass filter in a Sallen-Key topology. This topology was selected because of its simplicity and low part count. The second stage filter also has a cutoff frequency of ~14Hz to match the first amplifier stage and a gain of 10. Figures 9 and 10 show the schematic diagram for the active filter and the simulated frequency response respectively.

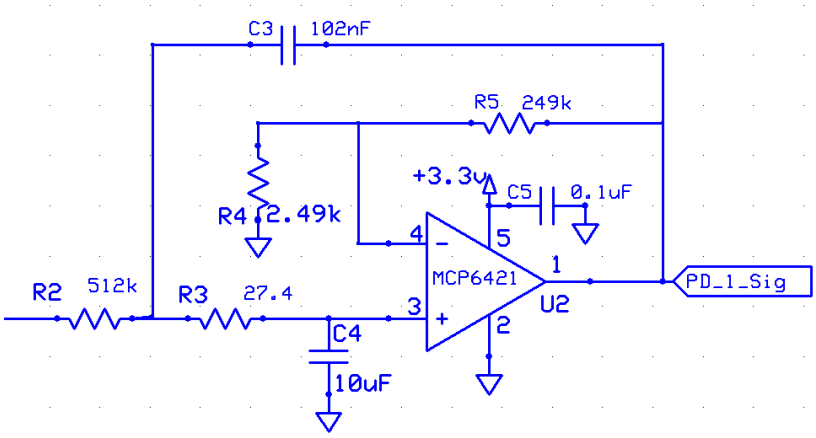


Figure 9: Secondary Gain Stage with Filter

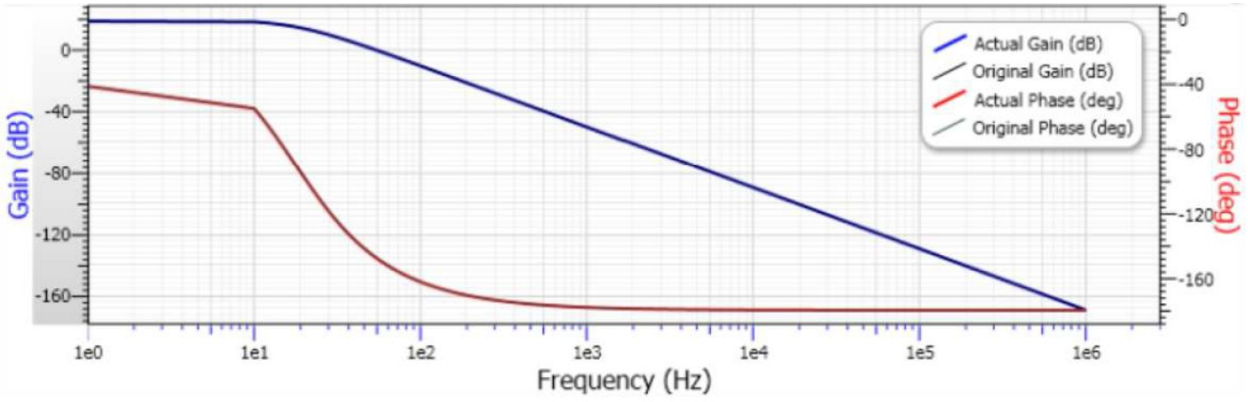


Figure 10: Filter Response

2.4.3 Microcontroller

The microcontroller interfaces with the amplifier and filter circuitry, the LED controller, the motor control relay and the PC running a simple user interface as shown in the block diagram in Figure 11.

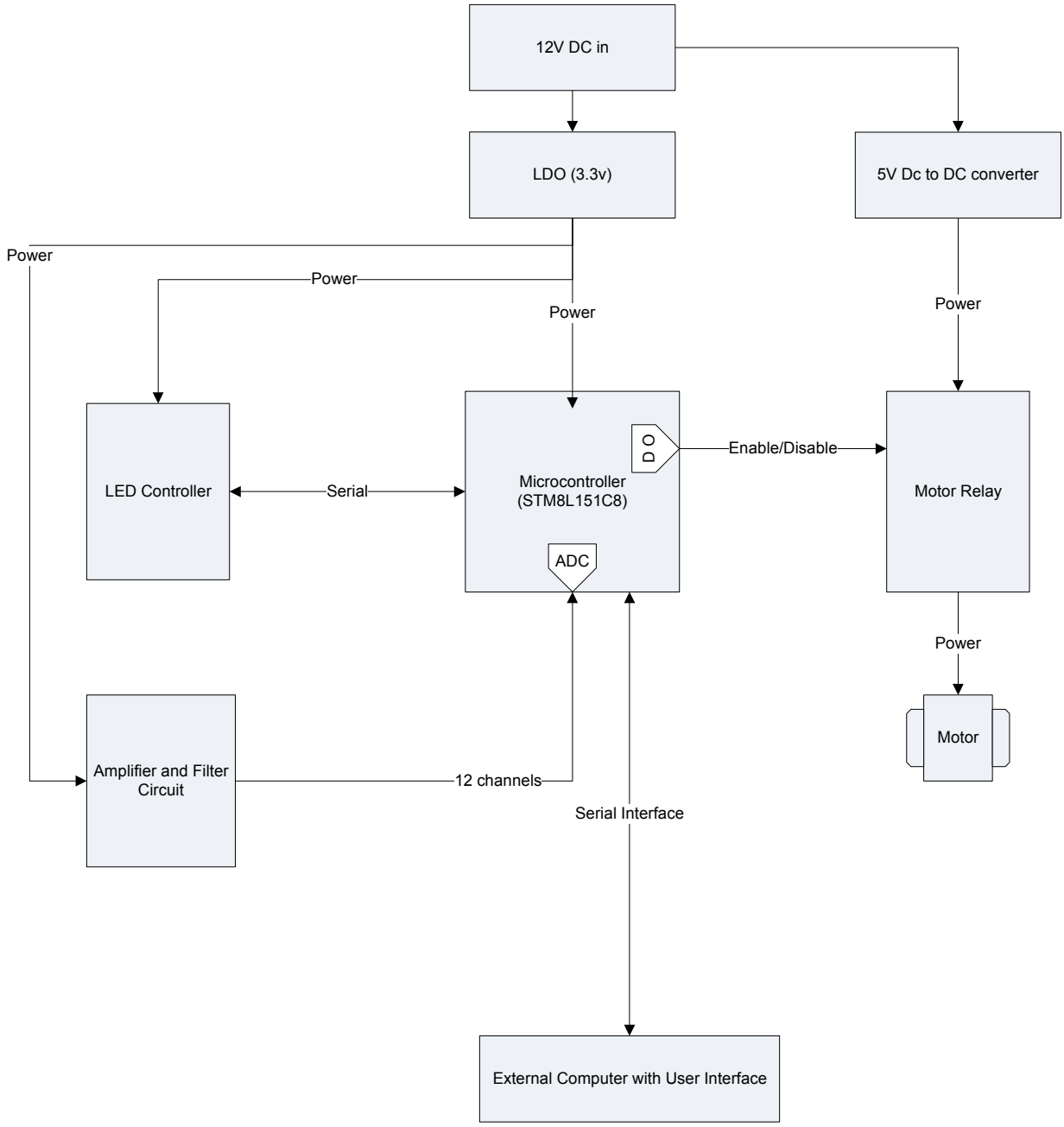


Figure 11: Control System Block Diagram

The STM8L151C8 microcontroller from ST microelectronics is appropriate for this application because it has 64K bytes of flash memory for firmware, 2K of

EEPROM for calibration constants and tables, a 25 channel ADC with 12 bit resolution, and a high precision internal oscillator. This eliminates the need for an external oscillator and an external analog to digital converter or analog multiplexer.

2.4.3.1 Firmware

The Firmware allows the system to be run in manual or automatic (free running) mode. A basic flowchart of the main function is shown in Figure 12. The main loop runs with a period of 100mS as timed by a timer interrupt service routine. The serial communication with the host PC is handled with a hardware peripheral within the microcontroller and double buffered within the serial interrupt service routine.

2.4.3.2 Initialization

On power up, an initialization function is called in which all the peripherals are initialized and all stations are scanned for faults. The stations are read with the LEDs off for all samples and the ADC values are sampled and filtered. The sampling and filtering function reads 64 sequential samples (with no delay between them) and calculates the median16 points. Those 16 points are averaged together, resulting in a single data point for that sample. The median filter serves to remove signal outliers from the data which would otherwise affect the average. The data point is then compared to a maximum threshold. A

value exceeding the threshold indicates a light leak, an open door or a faulty analog signal path. The stations are also scanned with their respective LEDs on and the ADC values are compared to a minimum threshold. A value that is too low indicates either a blocked optical path or a fault in the analog signal path. A value between two intermediate thresholds indicates that a sample is present in the station. Error conditions are transmitted to the host PC via the serial interface and the locations associated with errors or samples installed are recorded in a global array.

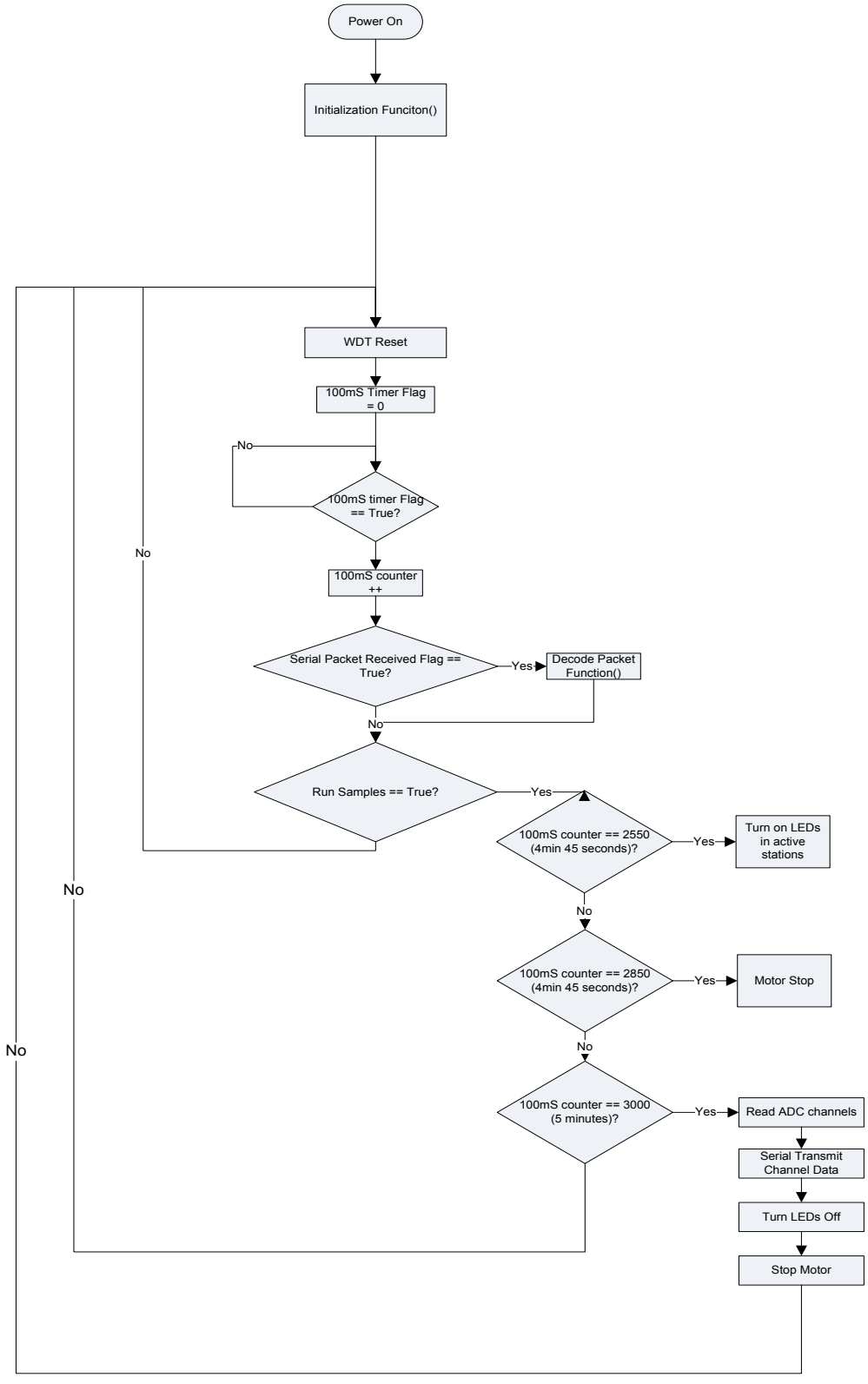


Figure 12: Software Functional Flowchart (Level 0))

2.4.3.3 Communication

After the initialization function is executed the firmware runs the main loop. Serial communication is handled asynchronously so as to not affect the timing of the measurements. When a complete serial packet is received a new-packet flag is set. Within the main loop, every 100mS, the new packet flag is polled. If a new packet is received via the serial port, then a packet decoder function is called. All the serial communication is operated at 115200 baud with an 8-N-1 parameter setting. Packets must begin with an open bracket '<' and end with a closed bracket '>'. Any characters not encapsulated within the brackets are ignored. A list of recognized commands is shown in Table 2.

Packet	Function	Returns
<S>	Scans all positions for samples	Sample positions
<P,0xaa, 0xbb>	Sets LED aa to intensity bb	OK
<R>	Read all stations	Returns filtered ADC value of all 12 stations
<M,0xaa>	Motor Run or Stop. aa = 01 for run, 00 for stop	OK
<G,0xaa>	Run Procedure	OK
<N>	Auto Run	OK

Table 2: Serial Command Packets

2.4.3.4 Sampling

If the system is in Auto Run or Run mode, the firmware will compare the 100mS counter to preset values to update the test state machine. The sampling cycle state machine comprises 4 states, as shown the Figure 14. State 1 occupies

most of the cycle time and serves to agitate the samples to encourage bacterial growth. The LEDs are turned on in state 2 in order to allow sufficient time for them to warm up before the samples are read. This is necessary to eliminate the effect of chromatic variation due to LED die temperature and the duration was determined empirically. Because moving liquid within the sample vessel will refract light in unpredictable ways during the sampling window, the motor must be stopped for 15 seconds in state 3. State 4 is the final state in which the samples are read by the ADC and processed with the digital filter described in section 2.4.3.2 before the results are transmitted to the host PC where they are logged.

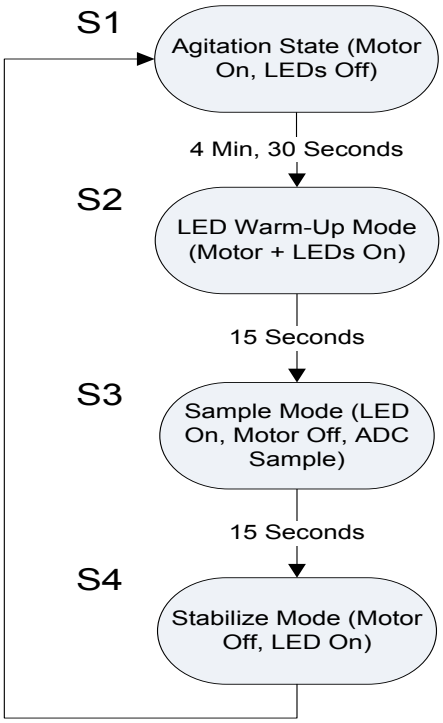


Figure 14: Sampling State Machine

2.5 Incubation System Design

In order to maximize growth of microbial cultures which may be pathogenic to humans, it is necessary to maintain culture specimens in an environment which is held at or near human body temperature. The incubation system under design therefore, will have an incubation target temperature of 35.0 degrees C. The two most common methods of incubation are contact incubation (maintaining the specimen tube in a directly heated block) and conditioned incubation (maintaining the air inside an incubator cavity at the target temperature, and using air as the heat transfer mechanism). To implement a contact incubation heater in this design, the heating element would need to be in contact with the sample holding block, which must be allowed to move during agitation. That would require high current conductors within the flex cable which would increase the system cost. Instead, this design utilizes a conditioned incubation system because it results in a system with the lowest cost and highest reliability while still achieving adequate temperature control for bacterial growth. In this system, a fan-driven air column will be forced over the comparatively large surface area of an aluminum heat sink at a continuous rate. The heat sink is warmed by a resistive load at a controlled rate, based on feedback from an air temperature sensing device located downstream of the heated air inside the incubated cavity. A simplified block diagram of the incubation system is shown in Figure 15 below.

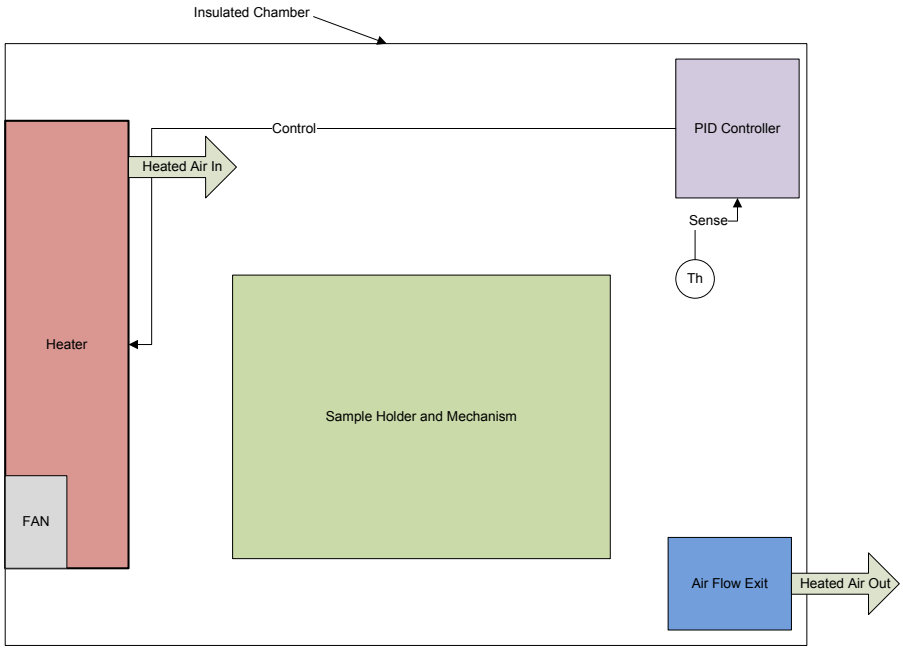


Figure 15: Incubator Design

2.5.1 Heater Design

The power capacity of the system heater has to balance the minimum power needs of the incubation system with other factors such as response time and cost. In estimating the power required to incubate the chamber, several assumptions were made. Firstly, it is assumed that the instrument is operating at steady state and that the incubated cabinet is heat soaked at a temperature of 35 Degrees C. Secondly, it is assumed that the instrument is being loaded with the worst case load of 12 inoculated culture tubes. Thirdly, it is assumed that each tube contains 15cc of fluid (which is approximated to have a thermal mass of water). Fourthly, it is assumed that the 12 tubes being loaded into the incubator are at room temperature (25 degrees C) and at atmospheric pressure. Lastly, it is

assumed that in order to provide the required performance the incubator must be capable of raising the 180cc of water (the combined sample volume of the 12 tubes), 10 degrees C (from 25C to 35C) in less than 5 minutes (1 sample period). The energy required to raise 1cc of water 1 degree C, is 4.18 Joules. This system requires 180CC of water to be raised by 10 degrees C. This yields a product of 7,524 watt seconds. Given that we have allowed 5 minutes for the tubes to come to temperature (7524 watt seconds / 300 seconds), the system heater must be capable of delivering a continuous 25.08 Watts at an absolute minimum

The system heater nominal power requirement was derived using a presumed room temperature of 25 degrees C. It is possible and perhaps even expected, that the instrument will operate in temperatures as low as 10 degrees C in locations with sub-optimal indoor temperature control. Given the system need for responsive proportional control under these conditions, the likelihood of occasional user door openings, and the recognition that the insulation of the incubated cavity is imperfect, the system was designed to deliver 3X the nominal power requirement.

The heater consists of TO-220 power resistors affixed to a finned aluminum heat sink using a thermally conductive compound to facilitate good heat transfer. The heater is powered by a 12VDC source and has a total resistance of 2 ohms, for a calculated heater capacity of 72 watts.

A more common heater construction would utilize a ni-chrome wire, but that design has been ruled out because it would radiate at wavelengths which would pollute the optical measurement system. When deployed in areas where the ambient temperature is expected to be above the incubation target temperature of 98C, the system would require the use of a Peltier Thermo-electric Cooler (TEC) in place of the resistive heater. The TEC would allow the blood culture machine to operate in both lower ambient temperature environments where the TEC would be operated as a heater, and in higher ambient temperature environments, where the TEC would operate to cool the chamber. The PID controller has additional control signals which would allow it to provide seamless control across the transition point between heating and cooling in future design iterations. Because the prototype was developed and tested in an area not subject to such a high temperature extreme and a resistive heater was already on hand, the TEC was not implemented in this prototype.

2.5.2 Airflow

Given that the incubation system heater will be controlled proportionally, the incubation system fan has been designed to be held at constant duty to minimize design and control complexity. A simple 12VDC electronic cooling has been selected to provide the incubation airflow. This type of fan is routinely used in electronic cabinet cooling applications, and is available from most major electronic parts distributors. The selected fan is brushless to maximize lifetime in a constant duty environment, and provides 14.5 Cubic Feet per Minute of flow.

With the estimated unoccupied cabinet volume of 1.1 cubic feet, the fan will facilitate 13.2 cabinet air exchanges per minute.

2.5.3 PID Controller

For the original system design, simple on-off temperature control functionality was included on the system main board. After considerable research on the temperature requirements of human pathogen physiology however, doubts arose as to the ability of such a system to maintain an adequately tight control band with such a design. As such, a low cost off the shelf industrial proportional integral derivative (PID) temperature controller was added to the system. The controller uses a type “J” thermocouple input to sense the cabinet temperature, and a pulse-width modulated logic output which is used to control the gate of the power FET that provides 12VDC to the system heater. The system target temperature may be set or changed from the front panel, and the actual cabinet temperature is displayed in real-time.

The P, I, and D characteristics of the controller may be manually tuned, or may be auto-tuned with a learning algorithm resident on the controller. The manual setting would allow a user to specify the proportional, integral and derivative terms used in the control algorithm. This could enable the system to reach the target temperature more quickly but it would also result in an increased temperature overshoot. The terms could also be changed to minimize overshoot, but the PID algorithm would then respond more slowly and take longer to reach the target temperature. I found that the automatic mode was able to control the

chamber temperature to within +/- 1 degree C within a short period of time, which is sufficient for bacterial growth. For this reason, automatic mode was used for the test procedure.

2.6 Agitation System

Bacterial culture requires readily available nutrients, appropriate gas-phase head-space, and favorable environmental factors. A stationary bacterial culture will allow growing organisms to quickly exhaust available nutrients, consume required gas-phase constituents, and render their local environment toxic with their own metabolic waste products. To redistribute nutrients and dilute local toxins, bacterial cultures are subjected to physical agitation during culturing. Agitation in this system is provided by reciprocal displacement of the specimen tube over a stroke of approximately 1 inch, and at a rate of approximately 2 cycles per second. The result will be a wave inside the specimen tube which mixes the tube's contents efficiently for a minimal input energy cost.

2.6.1 Head-space to Broth Ratio

Given that exposure to the gas phase components are important to promoting growth, the fluid-air exchange surface is maximized by holding the tube in a near horizontal position during agitation. This maintains the highest effective head-space to broth ratio. It conveys the additional benefit of periodically exposing the gas-permeable sensor in the bottom of each tube to the head-space gas. This

improves the permeation rate of the sensor, and shortens the sensor's response time.

2.7 Agitation System Design

The agitation mechanism design consists of a tube rack, mounted on a pair of stainless steel rails, by a set of four oil-impregnated bronze bushings. The bushings are captive on the rails, and allow the entire tube rack to slide back and forth on the rails. During powered agitations the tube rack is driven by a 12VDC gear motor, with a cam and connecting rod. As the motor rotates, the connecting rod drives the agitator in a reciprocal fashion, providing the agitation of the tubes. A photograph of the side view of the agitation system is provided as Figure 16. The tubes load into the rack from the left, and the subassembly can slide in a reciprocal motion horizontally along the steel rods visible at the bottom.

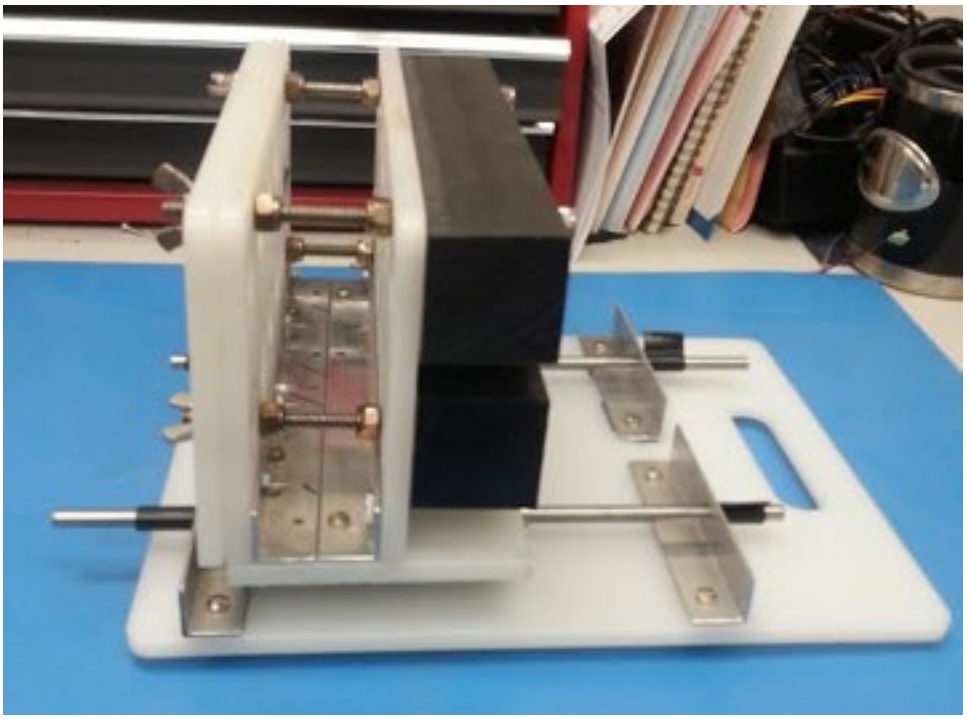


Figure 16: Sample Holder and Mechanism

2.8 Motor Control

While it is preferred to have agitation of the specimens be nearly continuous, agitation during data acquisition has the potential to induce optical and electrical noise, lowering signal to noise ratio. It will also be necessary for user to introduce new specimens, or remove specimens from time to time. In order to facilitate machine loading and to ensure that the tubes are stationary during measurement, agitation must be under machine control. A stop command will be issued to the agitation system prior to the start of data acquisition. The system will issue a start command to the agitation system after acquisition is complete.

3 Detection Algorithm

Given the clinical importance of sepsis, rapid detection of positive cultures is critical to the prognosis of a patient with a blood borne bacterial infection. The faster a culture can be detected, the higher the probability of patient survival. Prevention of false positive detection is also important, as it prevents the clinical laboratory from investing time, effort, and money that could be better utilized elsewhere.

Developing an algorithm to discriminate between positive and negative cultures would be a relatively simple undertaking if the entirety of the culture data were available for interpretation. But in order to provide rapid detection of positive cultures, interpretation of data must be made in real-time. With each incoming data point, the data must be scrutinized for features which indicate growth.

3.1 Processing Window

To minimize the impact to system resources, the data is stored in an array and interpreted with a sliding function. Each incoming data point is appended to the front of the array and the processing window slides across the data as new data becomes available. The result is a moving processing window. The processing window is defined by the most recent 60 data points in the array (which will subsequently be referred to as A_1 through A_{60}). As previously described, the sampling period is 5 minutes, so the processing window operates on 5 hours of

data. This window width was chosen empirically. It is possible that the blood culture instrument could process multiple arrays of multiple lengths in parallel.

3.2 Features of Interest for Discrimination

Given that the system under development is sensitive to CO_2 production, the signal features of both positive and negative blood cultures (and their impact on CO_2 production) have been considered. Although blood cells and bacterial cells both produce CO_2 as a function of respiration, there is a fundamental difference between the two in a sealed culture vessel. Blood cells are manufactured by the human body within the bone marrow and cannot reproduce on their own. Therefore when a specimen is taken from a patient and inoculated into a culture tube, the total number of viable blood cells present at the time of inoculation is at its maximum. Whereas if bacteria are present, they will replicate by binary fission, and as a result their number will increase exponentially. This difference has a significant impact on signal expectations.

Because the number of blood cells in a culture is already at its maximum at inoculation, the CO_2 levels from blood metabolism are expected to be increasing as measured by the system, but the rate of increase will be static or declining. More simply, blood is expected to produce a positive first derivative in the signal train (as a result of accumulated CO_2 from metabolism), but is not expected to produce a positive second derivative in the signal train (as the numbers of blood cells available to produce CO2 do not increase).

Conversely, the number of bacteria (if present) in a culture is at its minimum at the time of inoculation. And as the number of bacteria in the specimen increase as a result of binary fission, so too does the rate of their production of CO_2. Therefore, bacteria will produce a positive first derivative in the signal train (as a result of accumulated CO_2 from metabolism), but should also be expected to produce a positive second derivative in the signal train (as the numbers of bacterial cells available to produce CO_2 increase). Detection algorithms have been designed to discriminate this signal's increasing first derivative from its increasing second derivative. A theoretical example dataset representing a positive and a negative curve can be seen in Figure 17.

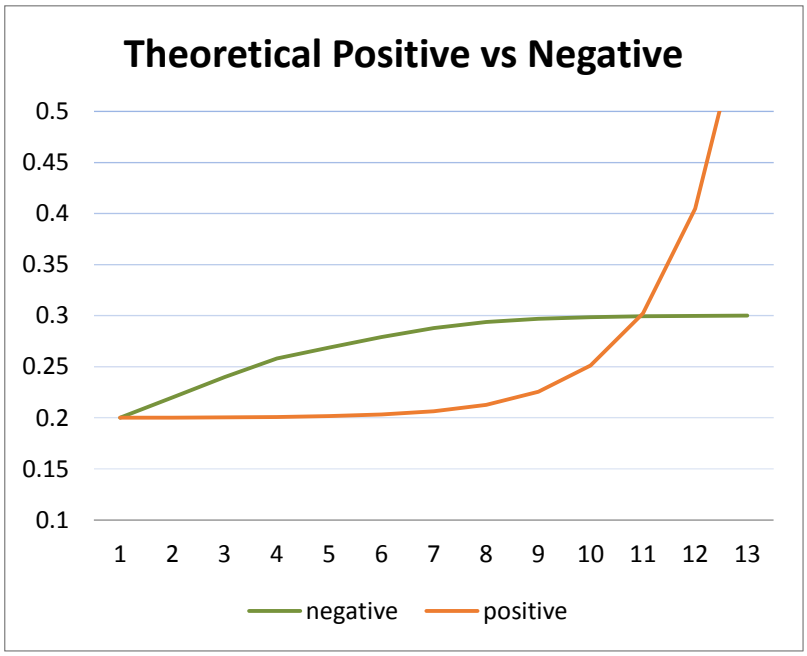


Figure 17: Typical Signal Traces

3.2.1 Algorithm Design:

More sophisticated methods of calculating 2nd derivative could be employed to detect growth in positive cultures, but such methods would provide greater tax on system resources, and are ultimately unwarranted. Simple calculations are employed here to accomplish the same ends. This algorithm calculates metrics on an array of 60 data points. For the purposes of this description the data points will be labeled from A1 to A60, with A60 being the most recent.

To provide some level of filtering, an average of the three most recent, three center, and three oldest data points in the array are calculated, and defined as NEWEST, and OLDEST.

$$\text{NEWEST} = (A58+A59+A60)/3$$

$$\text{TRUE CENTER} = (A29+A30+A31)/3$$

$$\text{OLDEST} = (A1+A2+A3)/3$$

The equivalent of the center of a linear approximation between the endpoints defined by NEWEST and OLDEST is then calculated.

$$\text{LINEAR CENTER} = (\text{NEWEST} + \text{OLDEST})/2$$

The algorithm then tests these calculated metrics to determine if positive second derivative is present in the data, and whether that positive second derivative is accompanied by a positive first derivative in the overall processing array. A

positive culture is declared if both tests are true. The following psuedocode demonstrates how a simple function can employ the discrimination algorithm.

```
IF      LINEAR CENTER > TRUE CENTER

AND

IF      NEWEST > OLDEST

THEN    Culture = Positive
```

A graphical representation of the intended function of the algorithm is included as Figure 18. It is only possible to satisfy the requirements of algorithm with data sets that have both a positive first derivative and a positive second derivative.

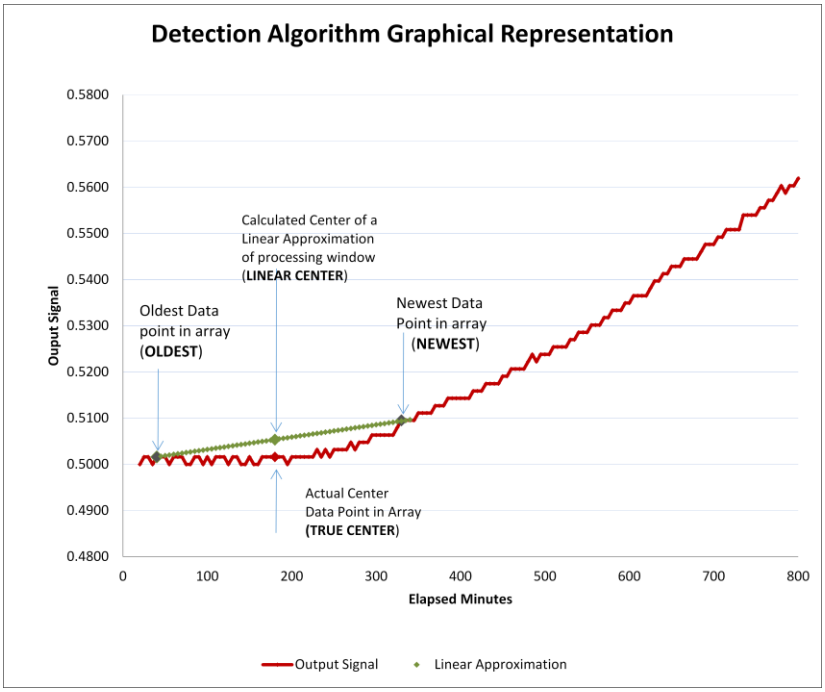


Figure 18: Detection Algorithm

4 TSB Broth Preparation

Medium was prepared using a traditional manual microbiology growth medium called Tryptic Soy Broth or TSB. This product is available commercially as a TSB Dehydrated Culture Medium (DCM) and was purchased from Sigma Aldrich as cat # 22092-500G. The medium was prepared by weighing out powdered TSB, and reconstituting in distilled water according to the instructions provided on the medium package insert. Once the TSB was fully dissolved in the distilled water, a syringe was used to dispense 10 ml of liquid TSB growth medium into each tube. A screw cap was loosely affixed to each tube after dispensing, and the tubes were placed in a metal test tube rack.

4.1 Sterilization of Media

If a steam autoclave were available to execute the sterilization of these tubes, it would have been utilized as the preferred method. In the absence of a steam autoclave, a pressure cooker was used. The rack of tubes (with the caps still loosely affixed) was placed inside the pressure cooker, and 2 cups of water was added to cover the bottom of the pressure cooker. The lid was affixed, heat was applied, and the tubes were held at 120 degrees C and 15psi for 15 minutes, as recommended in the TSB product insert. The pressure cooker was then removed from the heat, and allowed to stand until pressure dissipation. The lid was then removed, and the tube caps were tightened. This sealed the tubes at an elevated

temperature, drawing a slight vacuum on each tube as it cooled. . One tube was damaged during the pressure cooker sterilization process, most likely indicating that the tube came in contact with the water. That tube underwent an adhesion failure between the silicone sensor material and the glass side wall of the tube. As the tube cooled, liquid was pulled behind the sensor rendering it unusable. A photograph of the adhesion failure is included as Figure 19. This type of failure could most likely be avoided with the use of a proper laboratory grade autoclave.

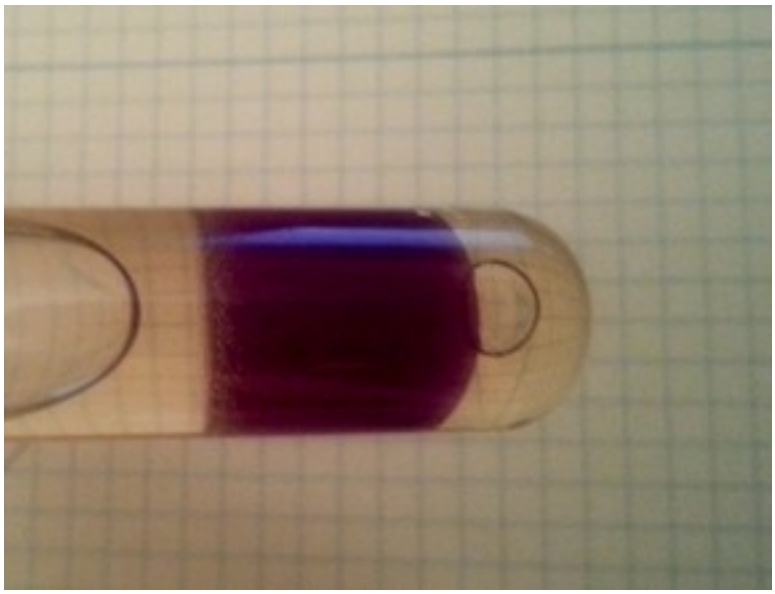


Figure 19: Adhesion Failure

4.2 Sterility Testing

The prepared media/sensor tubes were subjected to a sterility testing protocol using both the blood culture machine to evaluate signal, and a periodic visual inspection. Once each day, for the duration of a 7 day sterility protocol, each vial

was incubated, agitated, and tested in the blood culture instrument for one hour. The data was recorded and plotted for significant signal increases which would indicate bacterial growth in a contaminated tube. No signal trends indicative of growth were found. At the terminus of the 7 day protocol, the tubes were also visually inspected for turbidity, which would indicate bacterial growth. No turbidity was observed. Given the absence of growth indications, the tubes were declared sterile, and ready to be used in subsequent performance testing. The data for the sterilization of the prepared samples in shown in Figure 20. The relatively flat response at each measurement indicates no discernable change in color and so the samples are presumed sterile.

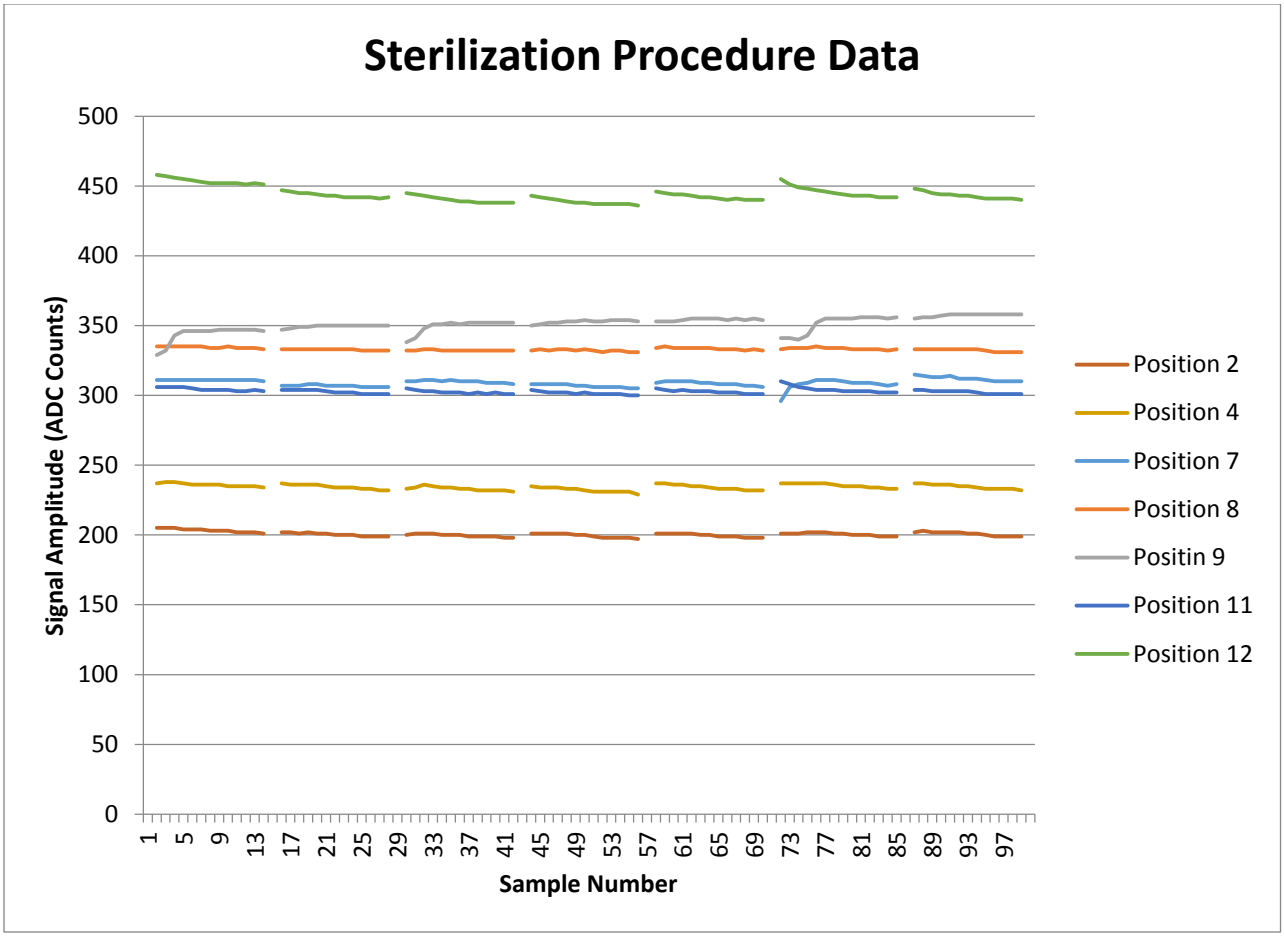


Figure 20: Sterilization Procedure Data

5 Performance Evaluation Protocols

5.1 Seeded Blood Culture Evaluation Protocol

A protocol was drafted for the performance evaluation of this Blood Culture Instrument, using seed cultures in a Microbiology laboratory (see Appendix D). This would allow for a direct comparison for the Time to Detection (TTD) for known standards with published TTDs for commercial systems. The protocol required the growth, quantification, inoculation, and detection of potential human pathogens. As such, institutional safety controls consistent with a clinical

microbiology lab (including personal protective equipment, fume hoods, sterilizers, etc. would be required. The original plan was to draw upon the facilities and experience of the University. Unfortunately, this path was not available, and an alternate simplified protocol was used.

5.2 Simplified Bacterial Detection Protocol

A simplified bacterial detection protocol was created, which affords the opportunity to evaluate the growth and detection characteristics of this system, without the use of a clinical microbiology laboratory. This protocol is included as Appendix E.

To summarize the intent of the protocol, bacteria are isolated from a cheek swab, and allowed to grow in broth medium. The bacteria are then standardized to a known quantity, using turbidity reference standards (McFarland Standards). Once standardized, tubes in duplicate are created for evaluation, and entered into the instrument in the configuration specified below in Table 3:

Specimen Type	Label	Machine Position
0.5 McFarland Inoculation	A (1)	7
0.5 McFarland Inoculation	A (2)	2
0.5 McFarland (with 10x dilution)	B (1)	8
0.5 McFarland (with 10x dilution)	B (2)	11
Negative Control	C (1)	4
Negative Control	C (2)	9

Table 3: Sample Position Map

After a period of testing for a minimum of 72 hours, data is collected from the instrument and subjected to signal processing and detection algorithm analysis. Data for each culture will be presented graphically, and the time to detection result plotted. The results will then be compared to the protocol acceptance criteria.

6 Results

The raw data for the six tubes of the protocol are plotted in Figure 21. Note that the difference between starting signals is somewhat variable owing to the geometric variations in optics, and to the surface variability of the sample tubes and LEDs. Given that system algorithms are designed to detect only relative signal changes, the distribution of starting signals is inconsequential to performance.

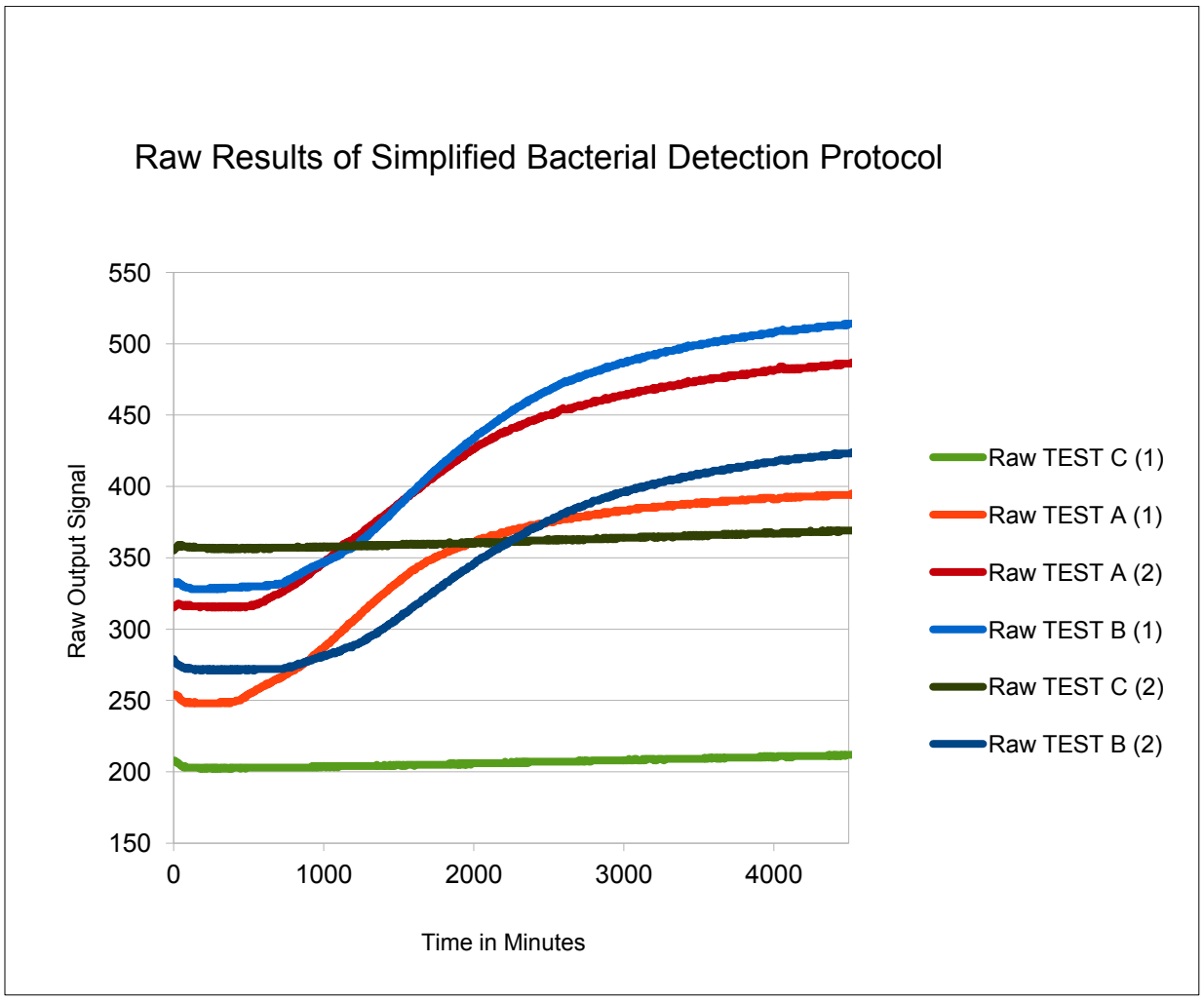


Figure 21: Combined Sample Data

For the purposes of understanding and explaining the relationship between the signals more easily, the data has been normalized to a common initial amplitude.

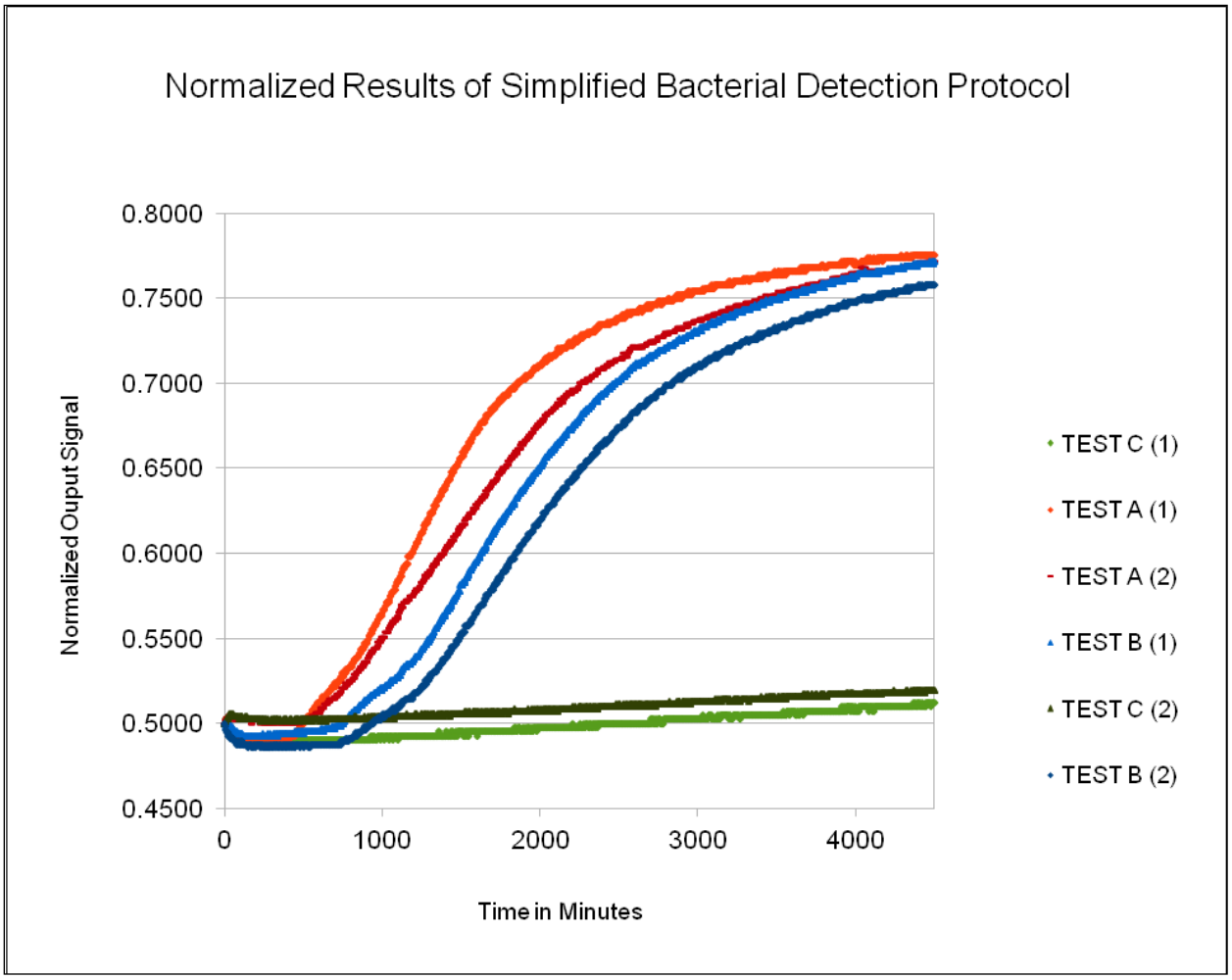


Figure 10: Normalized Data for all Samples

Normalization was accomplished by calculating a simple scalar for each culture based on the first data point of protocol, and adding that scalar to all subsequent points. In this case, a starting signal target of 0.5 was chosen, although this value

is arbitrary and has no impact on signal analysis or bacteria detection. As shown in Figure 22 above, the 6 protocol tube signal sets are more uniformly distributed and can be displayed on the same scale.

6.1.1 Detection Analysis

The 6 tubes of the protocol were subjected to detection analysis by the previously defined algorithm, in order to assure that the machine accurately differentiates positives from negative samples. The algorithm also provides a time-to-detection for each positive culture. The time-to-detection calculated by the algorithm has been compared to a graphical representation of each growth curve, to assure that detection occurs at a rational location on the curve. The time-to-detection has also been scrutinized to determine whether it satisfies the requirements.

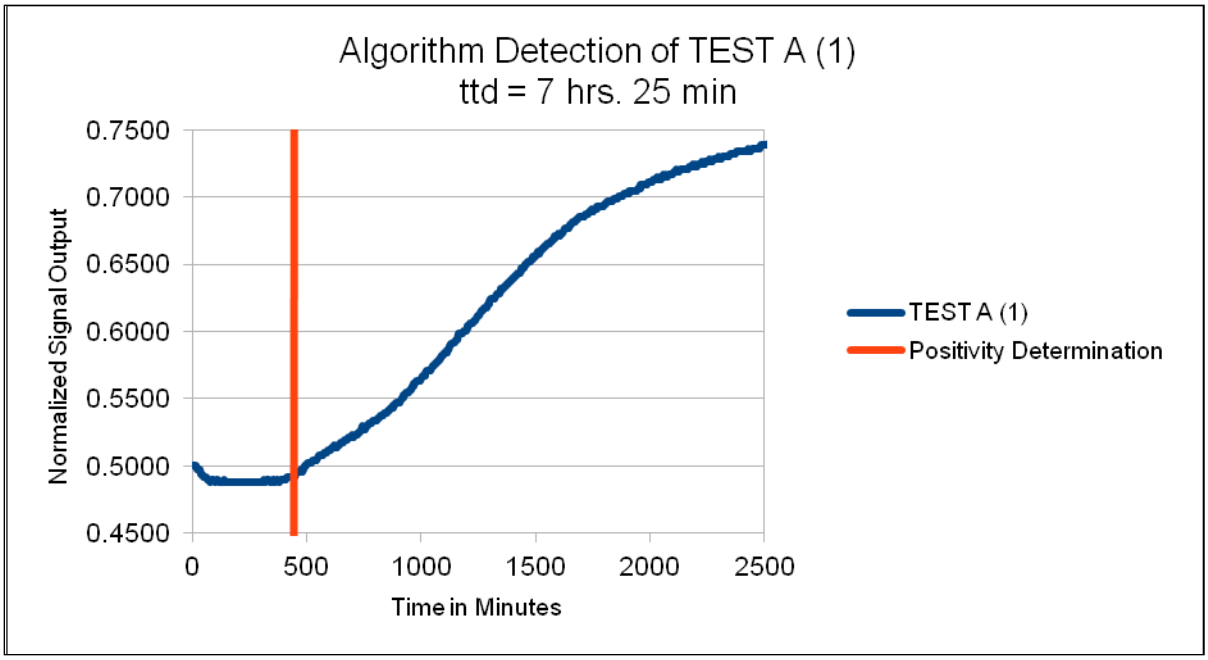


Figure 23: Algorithm Detection of Test A (1)

Figure 23, shows the growth curve data for TEST A, replicate 1of the protocol. The normalized instrument data is represented in blue, and the point of positive detection is represented as a red vertical line. A positive signal is determined at the point of intersection. This culture demonstrated robust growth, and was detected as positive at 7 hrs and 25 min. of elapsed protocol time. Note that the algorithm displays good sensitivity (detecting the culture relatively early in its expression of a positive second derivative).

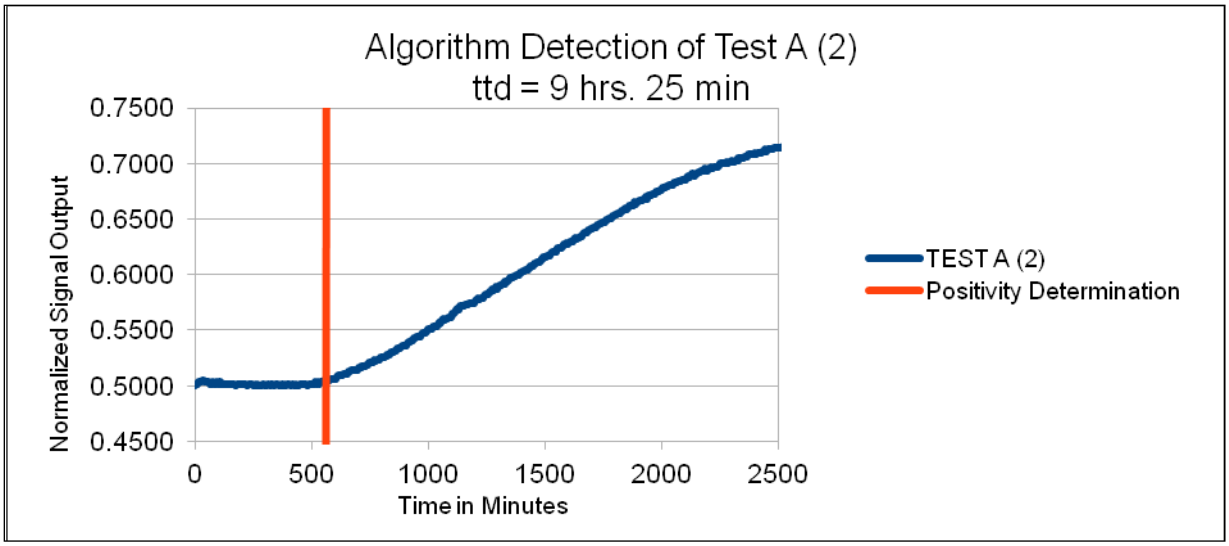


Figure24: Algorithm Detection of Test A (2)

Figure 24 represents TEST A replicate 2 of the protocol. Again the culture represents robust growth characteristics, and a time to detection of 9 hrs 25 min of elapsed protocol time.

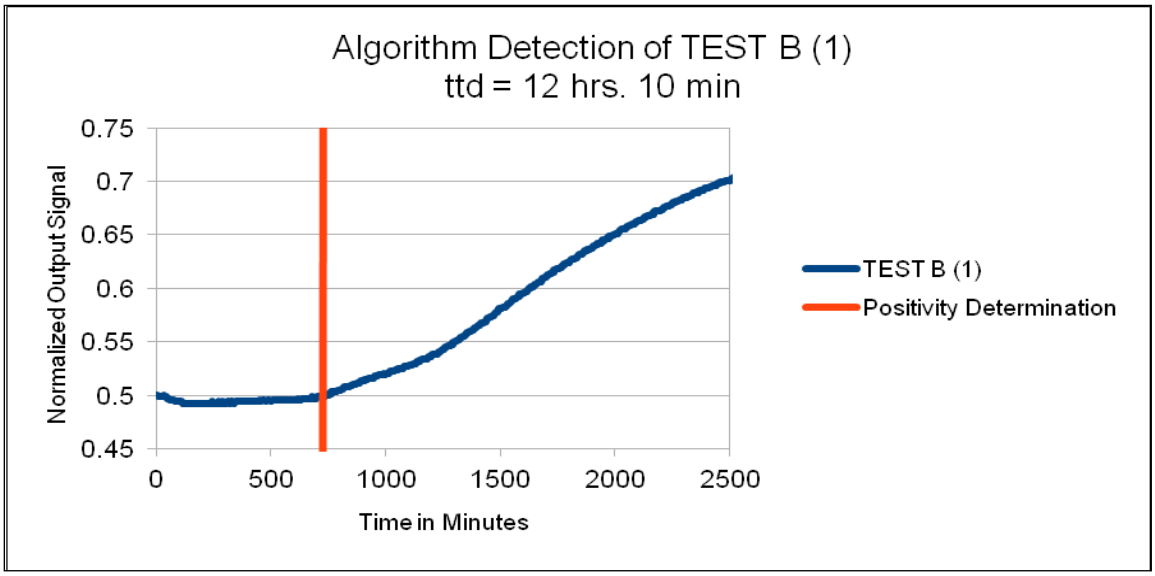


Figure 25: Algorithm Detection of Test B (1)

Figure 25 represents TEST B replicate1 of the protocol. Again the culture represents robust growth characteristics, and a time to detection of 12 hrs 10 min of elapsed protocol time.

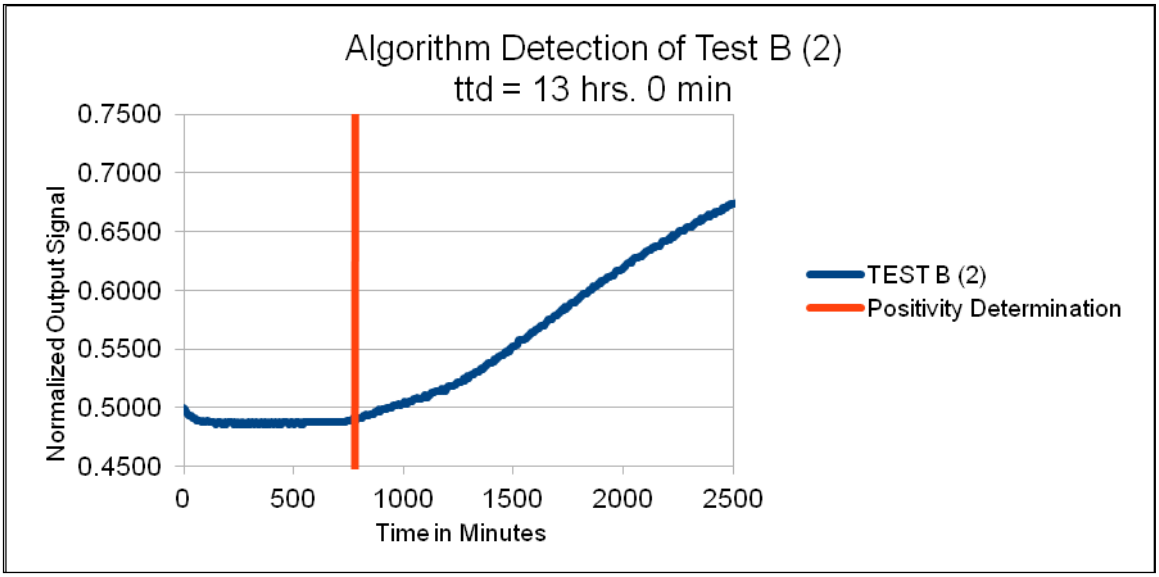


Figure 26: Algorithm Detection of Test B (2)

Figure 26 represents TEST B replicate2 of the protocol. Again the culture represents robust growth characteristics, and a time to detection of 13 hrs 0 min of elapsed protocol time.

The detection algorithm produces a predictable and reliable indicator of the point in the culture's growth phase, where the second derivative begins to increase. The two negative control cultures were not detected positive by the algorithm. The following table summarizes the results of the protocol.

Specimen Type	Label	Machine Position	Time to Detection

0.5 McFarland Inoculation	A (1)	7	7 hrs. 20 min
0.5 McFarland Inoculation	A (2)	2	9 hrs. 25 min
0.5 McFarland (with 10x dilution)	B (1)	8	12 hrs. 10 min
0.5 McFarland (with 10x dilution)	B (2)	11	13 hrs. 00 min
Negative Control	C (1)	4	No Detect
Negative Control	C (2)	9	No Detect

Table 4: Time to Detection for Tested Samples

6.2 Analysis of Time to Detection VS Concentration

Evaluation of the growth and detection results of the "Simplified Bacterial Detection Protocol to Evaluate Efficacy of a Low Cost Blood Culture Machine" demonstrates that the blood culture system performed as expected. The protocol replicates labeled "A" were expected to grow the fastest because they had initially the highest concentration of colony forming units, and yielded an average time-to-detection of 8 hours 20 minutes. The protocol replicates labeled "B" are a dilution of the concentration in tube "A", and would therefore expected to grow slower. Replicates labeled "B" yielded an average time-to-detection of 12hours 35 minutes. The tubes labeled "C" were negative controls, and as expected were not detected as positive. The difference in time to detection between sample tube A1 and A2 can be attributed to the variation in the volume of sensor in each tube. In tubes with larger volumes of sensor, the gas must permeate across a more silicone material before reaching the optical path. This

volume was not well controlled in the development of the prototype sensors, but with proper equipment in a manufacturing environment would be more consistent. The same can be said about the difference in time to detection between samples B1 and B2.

7 Conclusion

7.1 Performance

The stated design objective of this project was to produce a blood culture machine which would match or exceed the performance of existing devices, but at a cost low enough to make this technology available to markets which were previously not able to benefit from them.

The performance evaluation of this blood culture technology was not comprehensive. It was however, sufficient to demonstrate that the system faithfully detects the real-time production of CO2 by bacteria during the log phase of growth. Given that no existing technology can detect evidence of growth any faster than the CO2 is produced, it is reasonable to conclude that the performance of this instrument would be at least equivalent to commercially available devices.

There is the possibility (although this would have to be demonstrated clinically) that the performance of this system would actually exceed that of predicate devices. The agitation system design offers considerable advantage over existing devices, in that it provides more complete mixing and greater head-

space to medium exposure. The simplicity of the chemical sensor system should also allow for higher dynamic range and faster CO2 permeation rates than commercialized technologies. This should result in higher amplitude signals, with less delay, leading to faster detection.

7.2 Cost

7.2.1 Machine Cost

Given the difference in sample capacities of the various machines under consideration, cost comparisons were made using the metric of US$-per-Vial Test Location, or $-per-Station. Cost information was researched for 3 different BACTEC predicate systems. Manufacturing costs are trade secrets and therefore information was available for any of these systems. Commercial sales price, however, was obtained from the government website https://gsaadvantage-

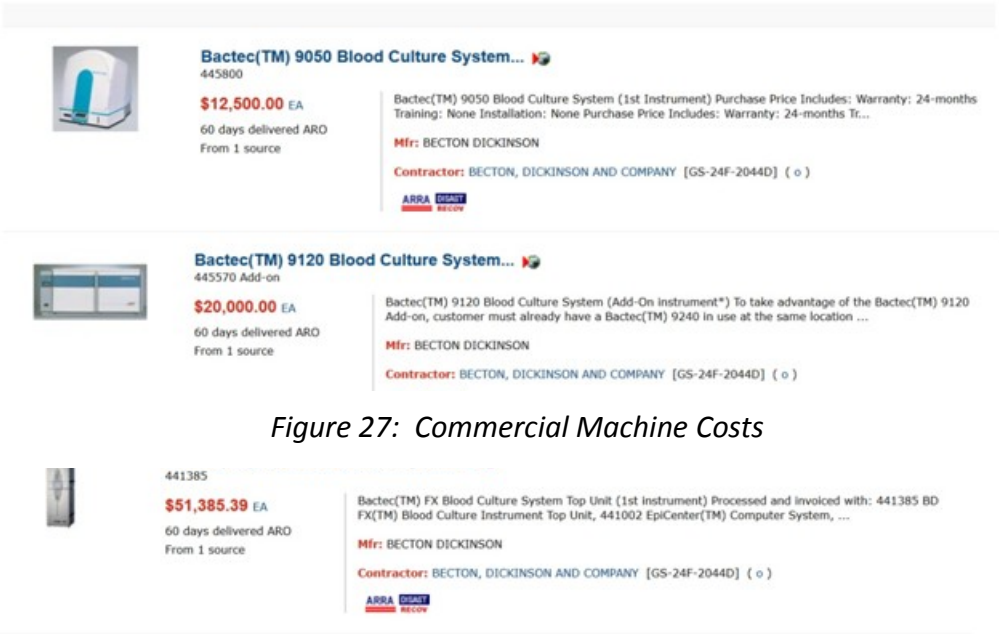


Figure 27: Commercial Machine Costs

test.fas.gsa.gov, a screenshot of which is in Figure 26.

To allow for direct comparison of systems, bill of material (BOM) costs, plus a labor, overhead and profit (LOP) estimate can be used to calculate a manufactured item's suggested sales price, based on a cost-plus markup approach.

The materials cost of the machine developed for this project are itemized in Appendix F and total to $284.21. Assuming offshore manufacturing, an LOP of 30% (industry standard) over BOM estimate is used to calculate the anticipated finished manufacturing cost for the machine. An additional 50% has been added for retail profit. This results in a total cost of $554.21. A comparison to existing devices is shown in Table 5.

Model	Machine Cost (Vials Not Included)
BACTEC 9050	$12,500
BACTEC 9120	$20,000
BACTEC FX	$51,385
Proposed Design	$554

Table 5: Price Comparison

Note that the sample capacity of the proposed design (12) is by far the lowest of all the machines. This explains in part the lower cost of the machine, but is a desirable design criteria considering that the expected number of patients served by rural clinics is much lower than the number of patients served by major hospitals. Also, this estimation is a worst case scenario as the machine has not

yet been optimized for design for manufacture and the prices listed do not include the disposable culture vials.

Although only in the prototype stage, the blood culture instrument as designed clearly satisfies the original requirement of equivalent or better performance, with a cost low enough to extend the reach of this technology into emerging markets traditionally beyond the reach of automated blood culture.

7.2.2 Disposable Cost

Assuming reasonable production volumes, disposable costs in a blood culture system are driven primarily by complexity and materials. This disposable was designed with low cost in mind. The culture vial is actually a standard test tube which is used in countless other platforms, and for general laboratory use. This allows the disposable to take advantage of volume pricing benefits which would not be available to an exclusive disposable design. Most commercial systems, on the other hand, require the use flat bottomed optical grade vials specifically made for the intended machine.

Also, the chemical sensor developed for this system benefits from a minimalist design, comprising only two parts; a silicone matrix, and a colorimetric pH indicator. This provides benefit in terms of material cost and reduced manufacturing complexity as compared to the commercially available systems. The fact that the sensor is interrogated in an axis perpendicular to the tube prevents the optical signal from being affected by the change in material properties of the liquid contents of the tube. This allows the sensor to be

fractionally transparent, resulting in high dynamic range, without the addition of optical barrier components in the sensor which are used by competitive systems.

8 Further Development

To develop this product beyond the prototype stage, the machine would have to be given sufficient industrial design attention to provide a commercially appealing product. This would be unlikely to add significant cost, as it would only include injected molded skins and bezels over a sheet metal incubation cabinet.

Detailed mechanical design attention would have to be spent on agitation and the vial rack. Automated manufacturing methods would have to be developed for the specimen vial to facilitate sensor fill and cure, in addition to media fill and capping.

9 References:

1. Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. *N Engl J Med* 2003; 348:1546–1554.

2. (November 2012) National Institutes of Health Sepsis Fact Sheet. Retrieved from http://www.nigms.nih.gov/Education/Pages/factsheet_sepsis.aspx

3. Czura, Christopher J., PhD and Distlerath, Linda, PhD. (November 2010). Sepsis poses life-threatening response to infection. Retrieved from http://www.mlo-online.com/articles/201011/sepsis-poses-life-threatening-response-to-infection.php

4. Babay, Emily. (2013, May 23). *Mid-Atlantic a hotspot for sepsis deaths, Penn study finds*. Retrieved from http://www.philly.com/philly/news/Mid-Atlantic_a_hotspot_for_sepsis_deaths_Penn_study_finds.html

10 Appendices

10.1 Appendix A: Predicate Systems

10.1.1 Manual Systems:

10.1.1.1 Septi-Check ®

10.1.1.2 Theory of operation

Septic-Check is a manual blood culture system comprised of a vial containing liquid growth medium, and a screw on top containing a solid culture plate inside a sealed chamber. Sample blood is collected directly into the liquid sealed culture vessel by venipuncture. The specimen is then incubated, and agitated to promote bacterial growth inside the liquid culture. Periodically (typically once every 8 hours, or once every 24 hours) the culture vial is inverted, washing the solid culture medium with whatever bacteria (if any) have accumulated or grown in the liquid culture medium. Bacteria deposited on the plate during a wash will grow visible colonies on the plate. The plate is read manually just prior to inverting the vial. Presence of visible colonies on the solid medium indicates a positive culture.

10.1.1.3 Utility

Septi-Check holds a significant market presence among laboratories which perform blood cultures, but which do not have sufficient blood

culture volume to justify the expense of an automated blood culture system.

10.1.1.4 Pros

High blood to medium dilution ratio provides good biological performance. The inclusion of a solid growth medium provides both a means of detection, and a ready source of pure isolated bacterial colonies for subsequent identification tests.

10.1.1.5 Cons

Disposables used in this method comprise many parts and are relatively complex, and are therefore relatively expensive (get price). The need for user interaction to read and plate-wash the cultures burdens the labs with labor costs.

10.1.2 Automated Methods

10.1.2.1 BACTEC® NR Series

10.1.2.2 Theory of operation

BACTEC® NR Series is a automated blood culture system comprised of an incubating cabinet, an orbital agitation system, and a gas phase IR measurement system. Sample blood is collected directly into a sealed culture vessel containing growth medium by venipuncture. The specimen is subsequently introduced into the incubator/agitator. Periodic monitoring (once every 8 hrs.) is accomplished

by transferring trays of cultures into a robotic machine which pierces the vial seal (septum) with a needle set, extracting a sample of the head space gas for analysis. The gas is pulled into an optical sample cell, and measured by IR fixed wavelength analysis to quantify the CO2 in the vial. Changes in CO2 or sufficiently high absolute quantities of CO2 are indicative of bacterial growth.

10.1.2.3 Utility

Once the only automated method commercially available, BACTEC NR technology has been supplanted by in-vial chemical sensor technologies owing to performance. This is likely attributable to cost and performance pressures.

10.1.2.4 Pros

BACTEC NR technology offered good biological performance (growth, but not necessarily detection). Published studies show good recovery of organisms, but not competitive times to detection for most organisms.

10.1.2.5 Cons

The inability to read specimens at a rate higher than once per eight hours provides a significant liability in time-to-detection of positive blood cultures. Continuous puncture of vial septum by the instrument carries a significant risk of vial cross-contamination, and operator pathogen exposure.

10.1.3 BACTEC® F Series

10.1.3.1 Theory of operation

BACTEC® F Series is a continuous-monitoring blood culture system comprised of an incubating cabinet, a rocking agitation system, and an optical measurement system. BACTEC employs a fluorescent chemical sensor technology capable of measuring O2 or CO2 with distinct chemical sensors for each gas. Sample blood is collected directly into a sealed culture vessel by venipuncture, and the specimen is introduced into the machine. Periodic optical monitoring is employed to detect changes in fluorescence of a sensor inside the vessel. The sensor is composed of a fluorescent indicator inside a silicone matrix. The silicone matrix allows the migration of CO2 or O2 into the sensor, while keeping the liquid phase components of the culture from interfering. Measurements are accumulated and interpreted for evidence of bacterial metabolism.

10.1.3.2 Utility

Originally the dominant market actor in automated blood culture, BACTEC has fallen to second. This is likely attributable to cost and performance pressures.

10.1.3.3 Pros

Performance of the BACTEC system is sound. Published studies show competitive times to detection for most organisms. User interface characteristics are generally sound. Media additives (particularly resin) provide a significant performance advantage in the presence of antibiotics.

10.1.3.4 Cons

Complex sensor formulation creates a relatively expensive disposable. Fluorescent measurement system requires optical filters for excitation and

emission for each measurement station. These factors in conjunction make it difficult for the BACTEC system to withstand the pressures of a cost competitive market. Rocking agitation system allows entrapment of blood biomass in the lower corner of the culture vessel, making it difficult to recover organisms encapsulated in that biomass.

10.1.4 BacT/ALERT®

10.1.4.1 Theory of operation

BacT/ALERT (Bio-Merieux) is a continuous-monitoring blood culture system comprised of an incubating cabinet, a rocking agitation system, and an optical measurement system. BacT/ALERT employs a pH-sensitive colorimetric chemical sensor technology. Sample blood is collected directly into a sealed culture vessel by venipuncture, and the specimen is introduced into the machine. Periodic optical monitoring is employed to detect changes in color, of a sensor inside the vessel. The sensor is composed of a pH indicator disc target inside a silicone matrix. The silicone matrix allows the migration of CO2 into the sensor, while keeping the liquid phase components of the culture from interfering. Measurements are accumulated and interpreted for evidence of bacterial metabolism.

10.1.4.2 Utility

Owing to a good balance of price and performance, the BacT/ALERT® system has emerged as the international blood culture market leader.

10.1.4.3 Pros

Relatively simple sensor design allows for a less expensive disposable than primary market competitor. Likewise, colorimetric as opposed to fluorimetric measurement system provides for a less expensive instrument design (no optical filters necessary). Smaller disposable provides higher sample density per unit machine volume. Biological performance satisfies market expectations.

10.1.4.4 Cons

Reflective measurement system geometry provides a significant passive background signal component minimizing system dynamic range. Smaller disposable compromises dilution ratio for sample density. Rocking agitation system allows entrapment of blood biomass in the lower corner of the culture vessel, making it difficult to recover organisms encapsulated in that biomass.

10.1.5 VersaTREK®

10.1.5.1 Theory of operation

VersaTREK (Thermo-Scientific) is a continuous-monitoring blood culture system comprised of an incubating cabinet, a stirring agitation system, and measurement system. VersaTREK employs a manometric technology. Sample blood is collected directly into a sealed culture vessel by venipuncture, and the specimen is introduced into the machine. Periodic pressure transducer monitoring is employed to detect pressure changes inside the vessel. Measurements are accumulated and interpreted for evidence of bacterial metabolism.

10.1.5.2 Utility

A relative late-comer to the blood culture market, VersaTREK has enjoyed steady market penetration owing largely to its biological performance advantage (see section 110.1.5.3).

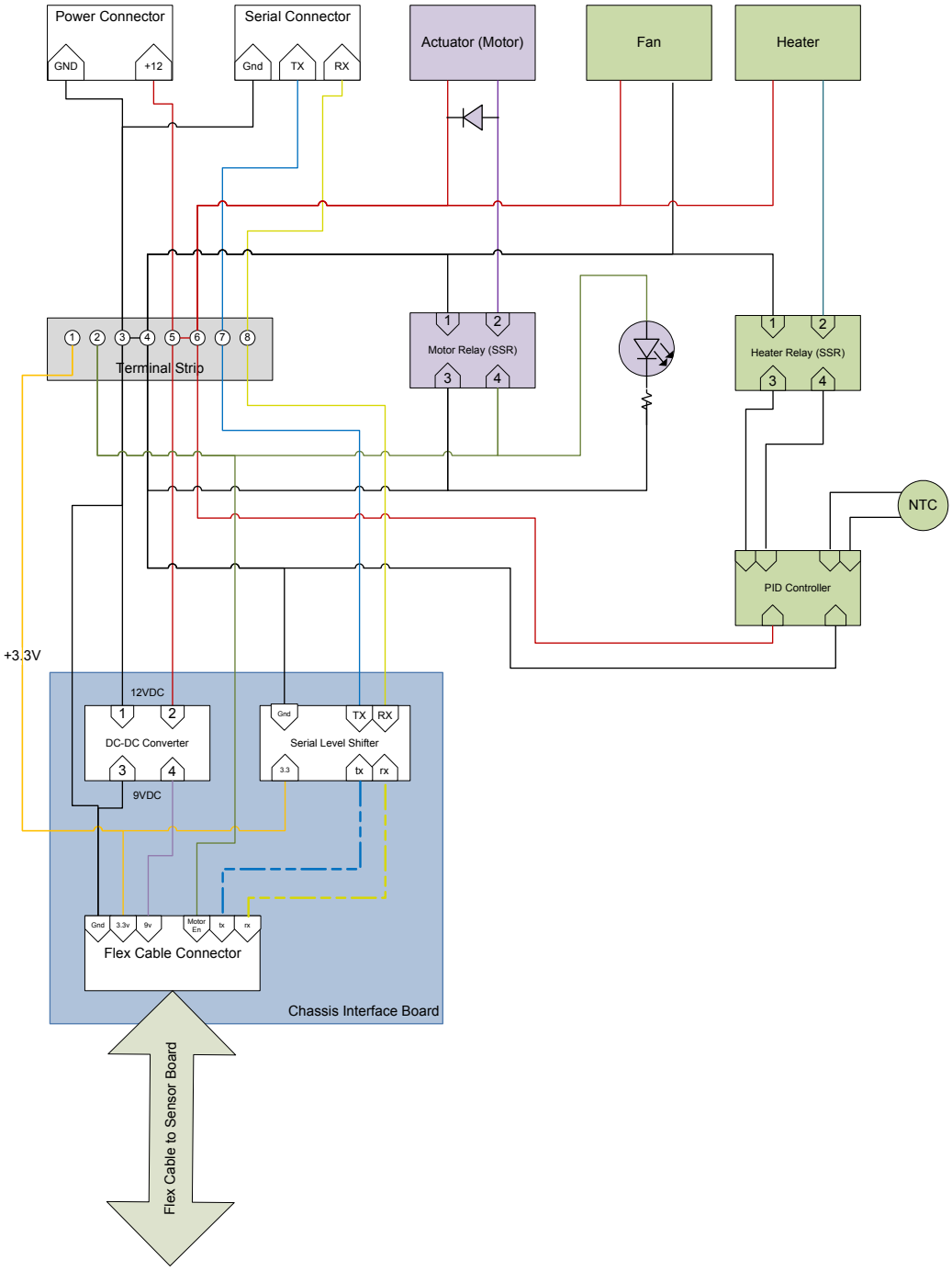
10.1.5.3 Pros

VersaTREK employs a comparatively large culture vessel, large media volume, and the highest blood-to-medium dilution ratio (1 to 9) of any continuous monitoring blood culture system commercially available. This high dilution ratio (coupled with vigorous stirring agitation) provides some of the best biological performance in the market.

10.1.5.4 Cons

The VersaTREK measurement system is based on a pressure measurement technology with significant limitations. While bacterial metabolism produces significant gas phase byproducts which may increase the pressure inside a sealed vessel, those same bacteria also consume gas phase metabolites. Depending on the organism, consumption and production rates may achieve a near balance making detection difficult.

10.2 Appendix B: Wiring Diagrams



10.3 Appendix C: Schematics

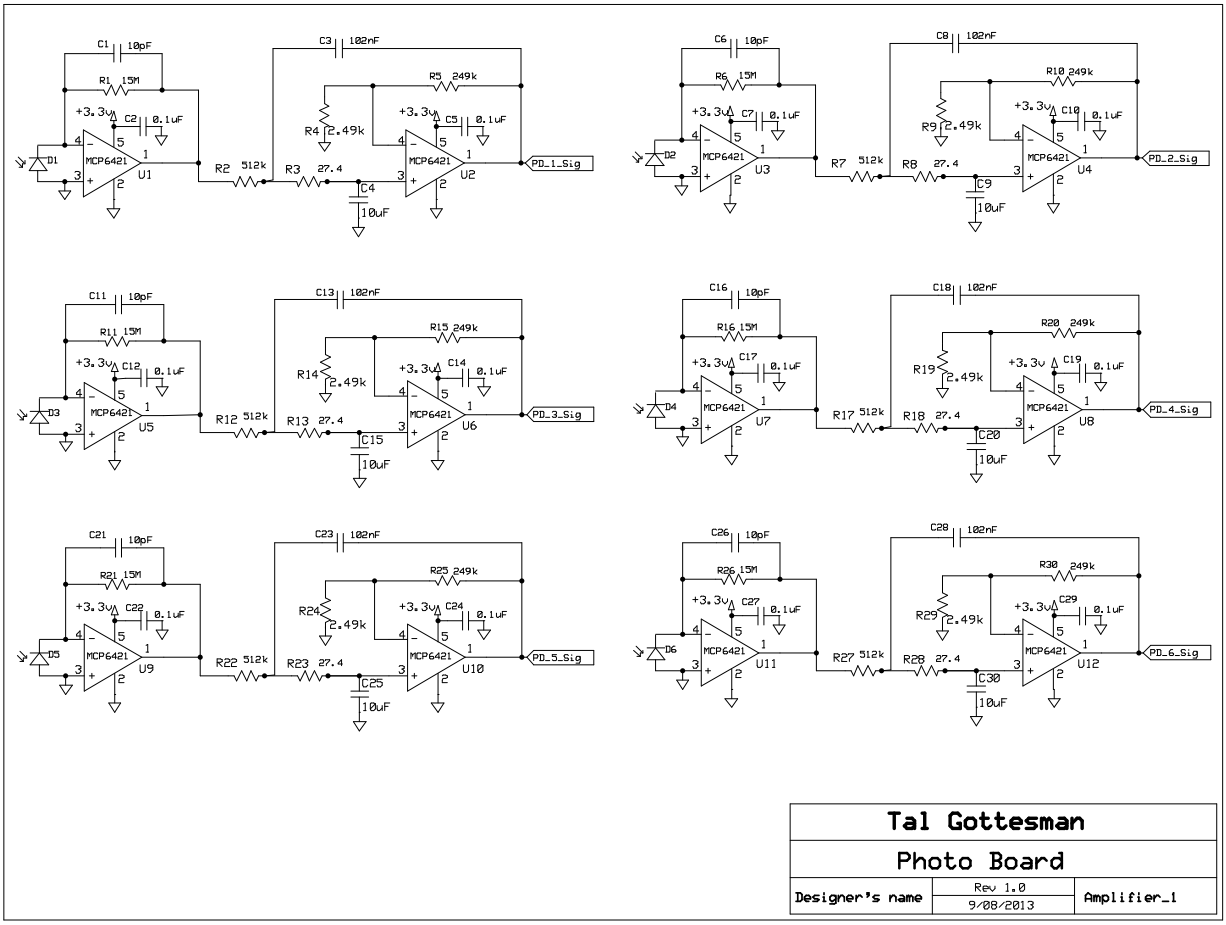


Figure 11: Amplifier and Filter Stage (page 1)

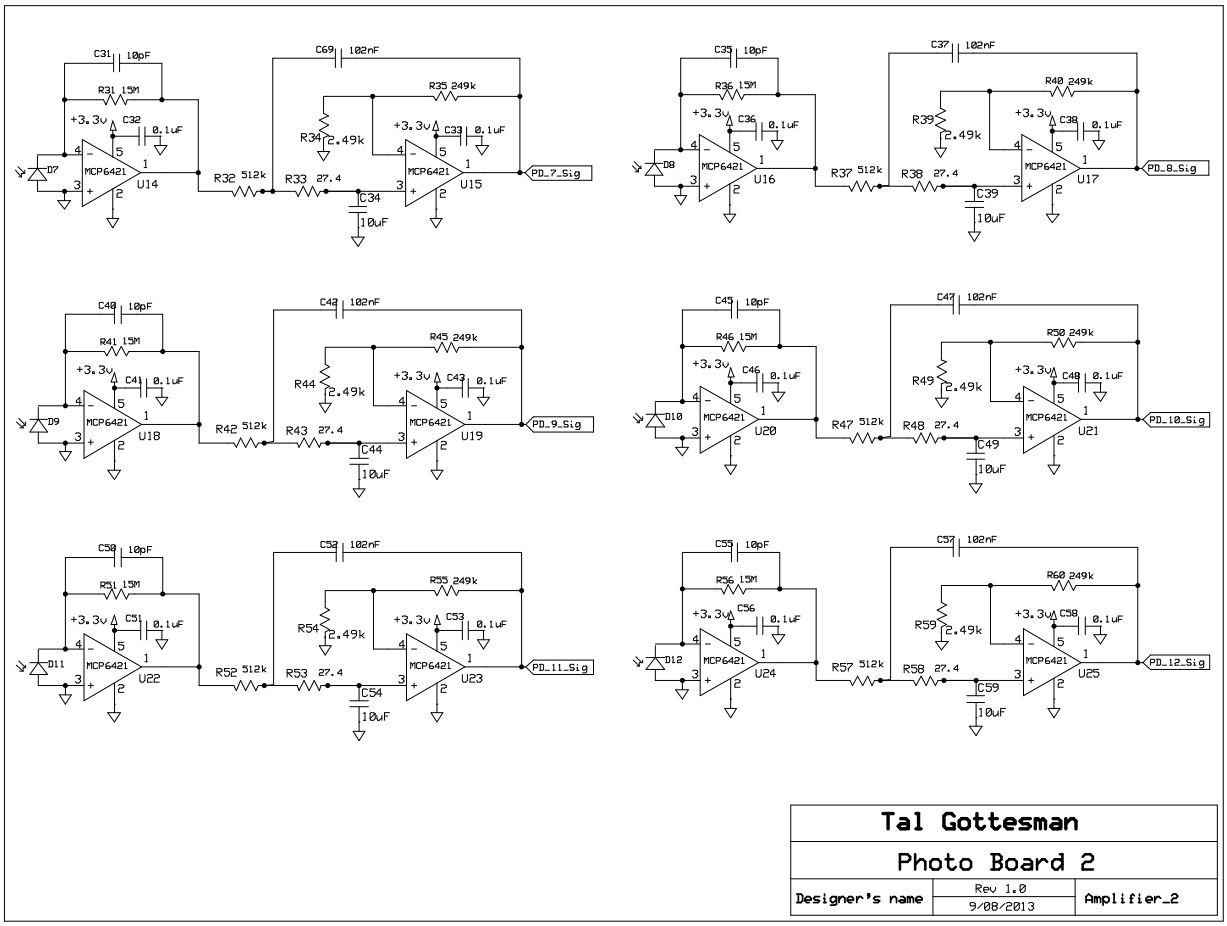


Figure 12: Amplifier and Filter Stage (page 2)

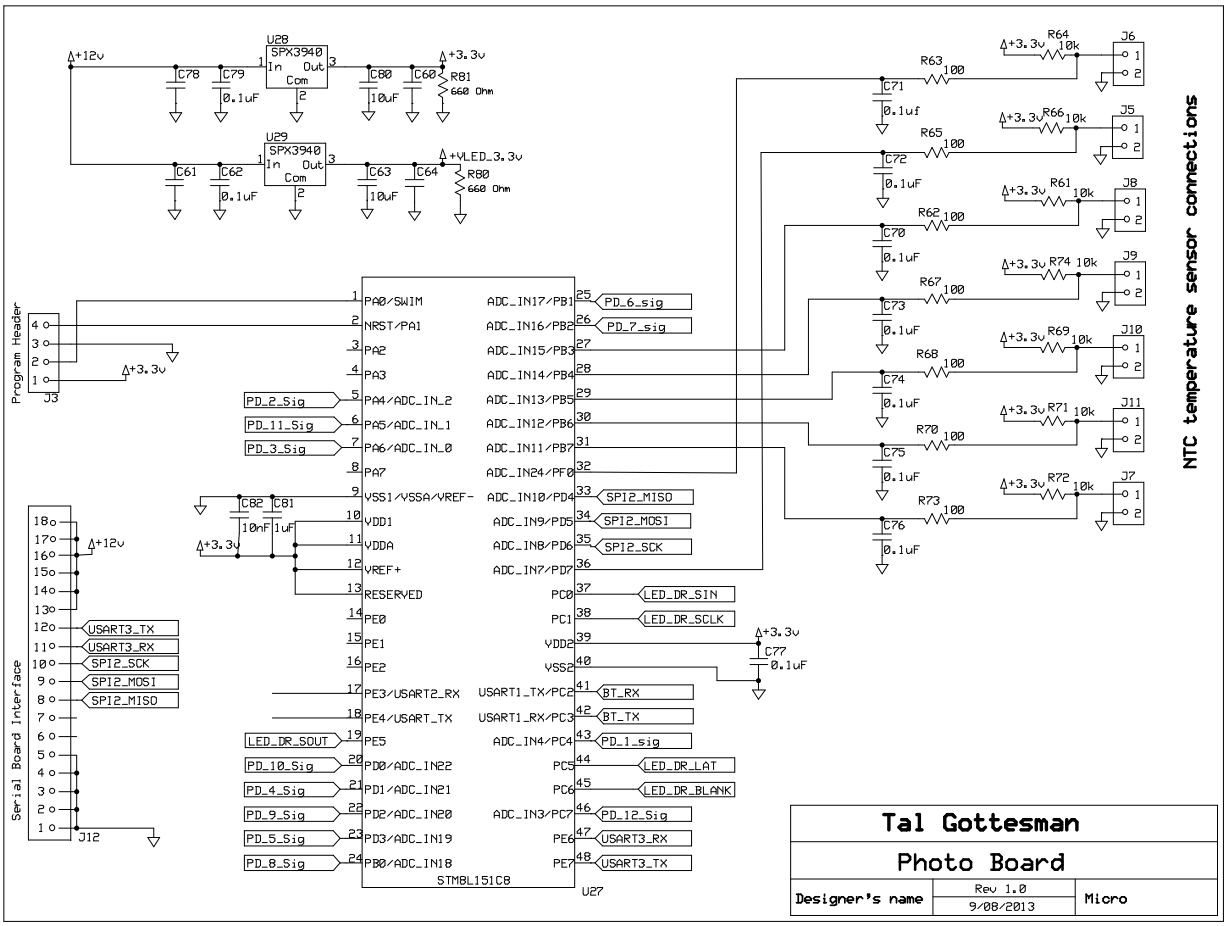


Figure 13: Microcontroller and Power Supply

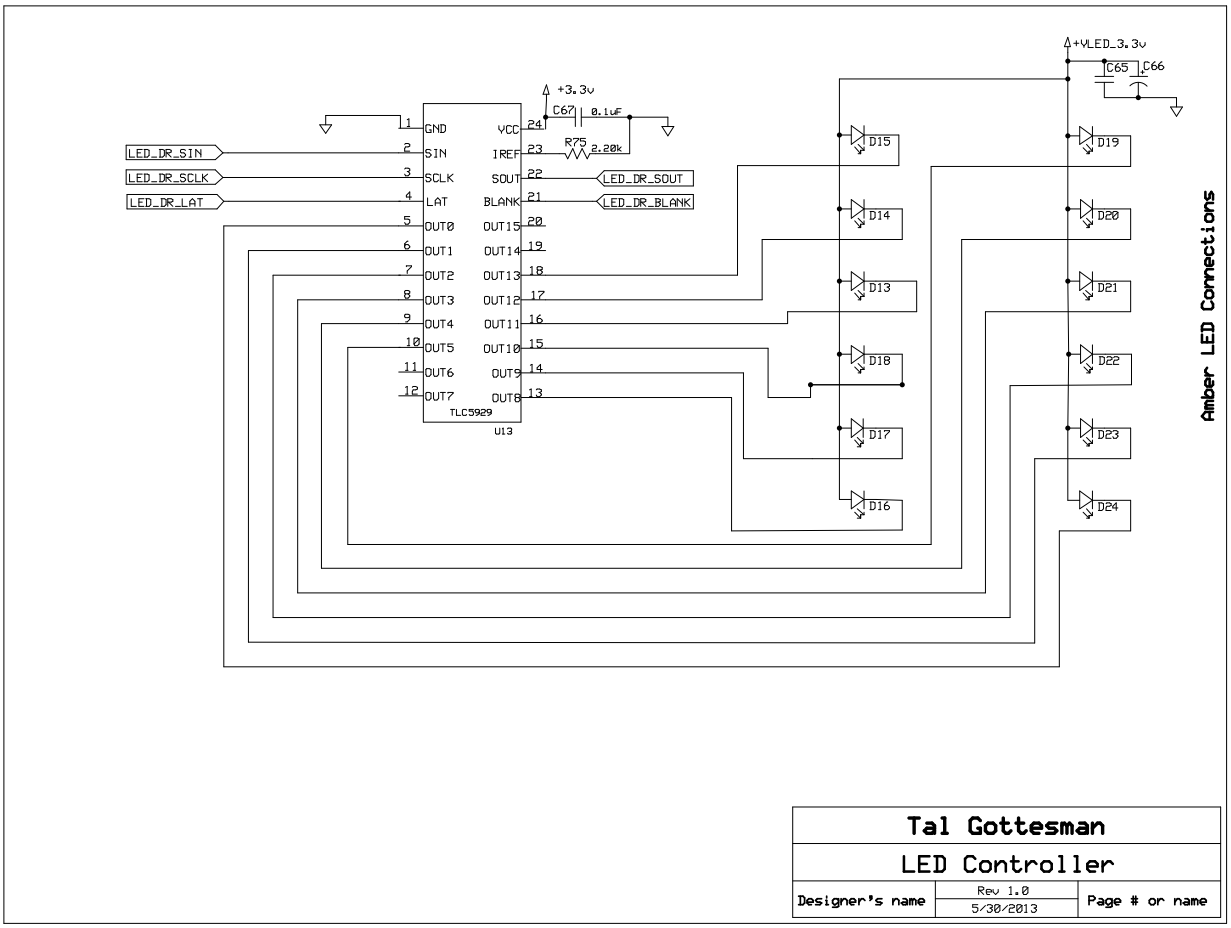


Figure 14: LED Driver

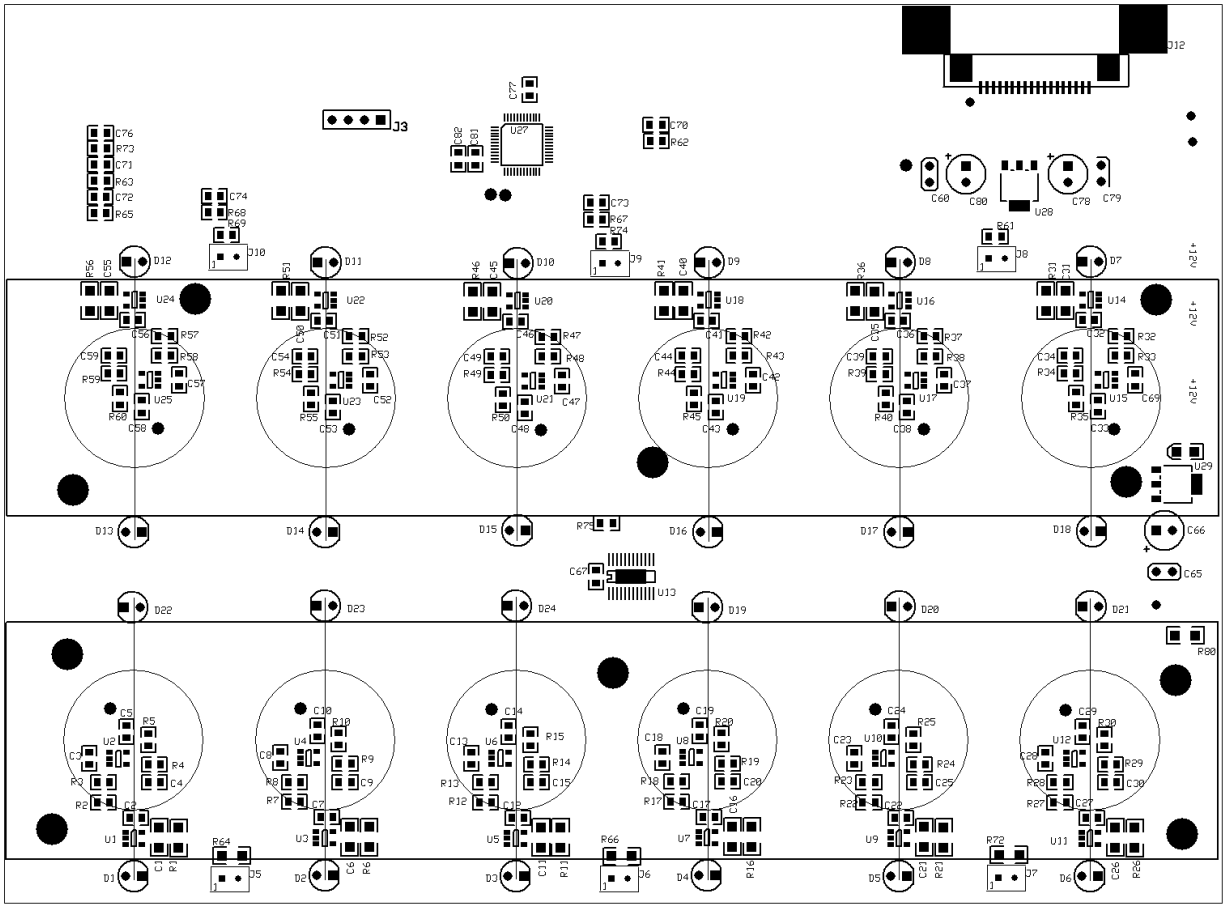


Figure 30: PCB Layout

10.4 Appendix D: SEEDED BLOOD CULTURE EVALUATION

10.4.1 PRINCIPLE

Currently many inspection/accreditation agencies require that prior to the routine use of a new culture method, the new test procedure should be evaluated against results from a reference method. This document outlines a simple procedure for evaluating the performance of a new method, allowing the evaluation to be performed with seeded suspensions versus patient specimens.

10.4.2 SPECIMEN

Normal, sterile saline containing an organism suspension of 10-100 CFU.

10.4.3 MATERIALS

1. Prepared vials of Experimental Aerobic Culture Medium with Sensor
2. Experimental Blood Culture Instrument
3. Reference blood culture medium expected Times to Detection
4. Test tubes of Normal, sterile saline - of sufficient size (to fit turbidity meter)
5. Test tubes of Normal, sterile saline - 10 mL
6. Plated Media Products appropriate for the organism: TSA with 5% sheep blood, Chocolate Agar, Sabouraud Dextrose Agar
7. 70% isopropyl alcohol pads
8. Sterile 1 mL pipettes for tube dilutions
9. Sterile 1 mL syringes for vial inoculation
10. Subculturing, staining, and identification supplies

11. Turbidity Meter to measure a 1.0 McFarland Standard
12. Sterile swabs
13. Organisms for testing. Examples of ATCC strains of common clinical isoisolates

Recommended Strains Are:

E. coli ATCC 25922

S. aureus ATCC 25923

K. pneumoniae ATCC 33495

10.4.4 PROCEDURE

IMPORTANT NOTE: Please be sure to follow Good Laboratory Practices and Universal Precautions at all times during the test procedure. All materials should be disposed of properly as required by your institution.

For most bacterial strains*, a McFarland 1.0 Turbibity Standard is equivalent to ~3 x 108
CFU(colony forming unit)/mL. Therefore, for most bacteria, a dilution of 106 (or three 1:100 dilutions) should result in a CFU of ~ 3 x 102 or ~3 x 10 CFU/0.1 mL inoculum (30 CFU).

1. Grow each organism to be tested overnight on the appropriate plated medium. Carefully examine and check each organism for purity. If there is a contaminant or the colonies have an unusual appearance, perform a Gram stain and/or identification to confirm the identity. If questions remain, do not use that organism at that time. Re-culture and repeat culture.

*NOTE: These dilutions are a guide and may need to be changed depending on the organism used to obtain the 10 to 100 CFU inoculum range. In the event that the colony count is below 10 or above 100, the experiment should be repeated with an appropriately adjusted dilution.

2. Add 10 mL of Normal saline to 4 test tubes labeled “A”, #1, #2 and #3. Note the organism name to be tested on each tube. Additional tubes should be labeled for each organism to be tested as indicated above.

3. Begin with the test tube labeled “A”. Take a sterile swab and touch a few well isolated colonies. Place the swab into the tube of saline and swirl. With the colonies added, the turbidity meter should measure approximately 1.0 McFarland units. If the meter does not read in this area, either: a) add more colonies if the reading is < 1.0; or b) dilute with sterile saline if the reading is > 1.0 to achieve the target 1.0 McFarland reading.

4. For a 10^6 dilution, add 0.1 mL of the standardized McFarland 1 organism suspension to the test tube labeled #1. Mix well.

5. For a 10^4 dilution, add 0.1 mL of the test tube #1 organism suspension to the test tube labeled #2. Mix well.

6. For a 10^2 dilution, add 0.1 mL of the test tube #2 organism suspension to the test labeled #3. Mix well. Tube #3 will be the inoculum used for the test and reference blood culture vials.

7. Plate Count - Prepare an agar plate of the final inoculum using the agar medium appropriate for each organism. Take 0.1 mL inoculum of tube #3 and inoculate the plate. Use the “spread plate” or similar sterile technique to evenly distribute the inoculum.

8. Wipe the tops of the vials with a 70% isopropyl alcohol pad. Label the test and reference blood culture vials with the organism’s name. Inoculate each test and reference blood culture vial (duplicate or triplicate if desired) with 0.1 mL of tube #3 of that organism.

9. Incubate the inoculated test in the instrument under test, and initiate a test protocol, facilitating the gathering of data, and the application of detection algorithms. Independently incubate the plate count media at 35oC.

10. Accurately record all results for both the test and reference blood culture systems used (i.e. organism CFU per vial, time to detection, plots, etc.).

11. Seed study performance for the selected organisms is relatively well characterized in predicate automated blood culture instruments. This allows for the inclusion of TTD performance expectations for these organisms. Evaluate data generated according to the following Recovery and Time-To-Detection expectations:

E. coli ATCC 25922	<11Hrs.
S. aureus ATCC 25923	<15 Hrs.
K. pneumoniae ATCC 33495	<12 Hrs.

Sterility Controls: Should be negative at the end of a 3 day test protocol

10.4.5 RECOMMENDATIONS:

1. Uninoculated sterility control vials and vials with added sterile saline (no organism), of both the test and reference blood culture vials, should be included.

10.4.6 LIMITATIONS

Care must be taken to prevent contamination of the vials during inoculation with the organism suspension. In the event that the colony count is below 10 or above 100, the experiment should be repeated with an appropriately adjusted dilution.

10.4.7 REFERENCES

Seeded Blood Culture Evaluation. Document MA-0100. Becton Dickinson Microbiology Systems

10.5 Appendix E: Simplified Bacterial Detection Protocol to Evaluate Efficacy of a Low Cost Blood Culture Machine

10.5.1 Purpose

As described in the original thesis project proposal, a low cost automated blood culture system would be developed and evaluated in a clinical microbiology laboratory. Seed cultures of known organisms (from the American Type Culture Collection) would be inoculated into sample tubes, with standardized concentrations. Time to Detection (TTD) for the seed cultures would be compared against the published TTD for commercially available units. This is the preferred scenario for the evaluation phase of the project because it allows for the direct comparison of the efficacy of the newly developed machine to the industry standard.

In the absence of access to a suitable clinical microbiology laboratory however, I propose using a traditional expedient bacterial culture method to evaluate the performance of the low cost blood culture machine instead. This document describes a simple protocol to establish the ability to incubate, grow, and detect viable bacteria from a cheek swab. Both methods are suitable for the evaluation of the low cost blood culture machine, but while the expedient method demonstrates the capabilities of the machine it does not allow for the quantitative comparison to commercial unit's TTD for a standardized seed culture.

10.5.2 Overview

Of the most common bacteria found in the mouth, *Streptococcus mutans* is the most numerous, and is a robust grower. The organism is facultatively anaerobic (meaning it will produce ATP by aerobic respiration if oxygen is present). It will therefore grow equally well in aerobic and anaerobic growth medium.

Given the robust growth characteristics of the organism and it's abundance in the human mouth, an aerobic culture from a cheek swab can be assumed to be dominated by *Streptococcus mutans.* This can be confirmed by identification later if necessary. This procedure will collect, standardize, and inoculate organisms into test specimens, to evaluate recovery and TTD for the automated blood culture system.

10.5.3 Materials

1 Set of 4 Prepared Barium Sulfate McFarland Standard tubes 0.5,1,2,4 (Fisher Scientific)

1 Wickerham Reference Card

1 Tube of prepared bacterial broth growth medium (TSB)

3 Tubes of 10ml sterile saline solution

1 Sterile swab suitable for specimen collection

1 Sterile transfer pipette

6 test target Automated Blood Culture Sensor tubes (with medium)

10.5.4 Procedure

1. Introduce the sterile swab into the mouth of the donor between the cheek and gum, spinning several times to facilitate saturation of the swab.

2. Remove the swab, and introduce the swab into a tube of prepared bacterial broth growth medium (TSB). Carefully agitate the swab several times, to ensure transfer of the swab contents into the broth medium.

3. Cap, and incubate the bacterial growth medium at a temperature between 35 and 37 degrees C for a minimum of 24 hours.

4. Observe the broth medium for indications of bacterial growth. (The tube should be cloudy and heavily laden with bacteria.)

5. Transfer 50ul of the bacteria laden broth suspension into a sterile saline tube.

6. Compare the turbidity of the saline tube to the turbidity of the 0.5 McFarland Barium Sulfate reference tube by observing the lines on the Wickerham Card for both tubes through the liquid column.

7. Continue to add bacteria laden broth in 50ul increments to the saline tube, until the turbidity of the saline tube matches the turbidity of the 0.5 McFarland reference tube when observed with the Wickerham Card.

8. When a turbidity level of 0.5 McFarland is achieved, the saline bacterial suspension will contain approximately 1.5X10^8 CFU/mL. CFU (colony forming unit) is a standard measure of bacterial density. For the purposes of this protocol, 1 CFU can be considered equivalent to a single organism. Label as tube A.

9. Transfer 1ml of the contents of tube A into a second sterile saline tube. Label this as Tube B.

10. Label the final unopened sterile saline tube as tube C.

11. Inoculate 50 uL of the contents of Tube A, into two Automated Blood Culture sample tubes. Label these tubes Test A.

12. Inoculated 50 uL of the contents of Tube B, into two Automated Blood Culture sample tubes. Label these tubes Test B.

13. Inoculate 50 uL of the contents of Tube C, into two Automated Blood Culture sample tubes. Label these tubes Test C.

14. Enter the 6 Automated Blood Culture sample tubes labeled A, B, and C into the blood culture machine, and initiate the automated testing sequence.

15. Collect data at the predefined interval for a period of 72 hours.

10.5.5 Expected Results

1. The Automated Blood Culture specimens labeled Tube A, should produce growth, and have an automated Time-To-Detection of less than 24 hrs.

2. The Automated Blood Culture specimens labeled Tube B, should produce growth, and have an automated Time-To-Detection of less than 30 hrs.

3. The Automated Blood Culture specimens labeled Tube C, should produce no growth, and should not be detected as positive.

The difference in TTD between samples in tubes labeled A and tubes labeled B will be a function of the growth rate of the organism, the sensitivity of the system, and the sensitivity of the algorithm, and will warrant further discussion and analysis. The data acquired by sampling the tubes labeled C will be used to test for sensor changes as a function of temperature and exposure to the TSB, as well as an indicator for contamination.

10.6 Appendix F: Costed Bill of Materials

10.6.1 Electrical BOM

Component	Value	Designator	uantit	Price	Totals	Part Number	Manufacturer	Distributor
Capicator	10pF	C1, C6, C11, C16, C21, C26, C31, C35, C40, C45, C50, C55	12	0.05	0.58	CL21C100JBANNNC	Samsng	Digikey
Capicator	0.1uF	C2, C5, C7, C10, C12, C14, C17, C19, C22, C24, C27, C29, C32, C33, C36, C41, C43, C46, C48, C51, C53, C56, C58, C62, C67, C70, C71, C72, C73, C74, C75, C76, C77, C79	34	0.05	1.67	C0805C104K5RACTU	KEMET	Digikey
Capicator	110nF	C3	1	0.47	0.47	C0402C112J5GAC7867	KEMET	Digikey
Capicator	10uF	C4, C9, C15, C20, C25, C30, C34, C39, C44, C49, C54, C59, C63, C80	14	0.11	1.57	CC0805ZKY5V6BB106	Yageo	Digikey
Capicator	100nF	C8, C13, C18, C23, C28, C37, C42, C47, C52, C57, C69	11	0.05	0.54	C0805C104K5RACTU	KEMET	Digikey
Capicator	DNP	C60	1	0.00	0.00			
Capicator	DNP	C61	1	0.00	0.00			
Capicator	DNP	C64	1	0.00	0.00			
Capicator	DNP	C65	1	0.00	0.00			
Capicator	DNP	C66	1	0.00	0.00			
Capicator	DNP	C78	1	0.00	0.00			
Capicator	1uF	C81	1		0.00			
Capicator	10nF	C82	1		0.00			
Photodiode		D1, D2, D3, D4, D5, D6, D7, D8, D9, D10, D11, D12	12	0.39	4.68	PD3333-3C	Everlight Electr	Digikey
LED		D13, D14, D15, D16, D17, D18, D19, D20, D21, D22, D23, D24	12	0.15	1.80	C503B-AAS-CY0B0251	Cree Inc	Digikey
Footprint		J3	1	0.00	0.00			
Footprint		J5	1	0.00	0.00			
Footprint		J6	1	0.00	0.00			
Footprint		J7	1	0.00	0.00			
Footprint		J8	1	0.00	0.00			
Footprint		J9	1	0.00	0.00			
Footprint		J10	1	0.00	0.00			
Footprint		J11	1	0.00	0.00			
Ribbon Cable Co	18 Positi	J12, J13	2	1.16	2.32	A100253CT-ND	TE Connectivity	Digikey
Resistor	15M	R1, R6, R11, R16, R21, R26, R31, R36, R41, R46, R51, R56,	12	2.03	24.41	CRHV1206AF20M0FKE5	Vishay Dale	Digikey
Resistor	100k	R2	1	0.04	0.04	RMCF0805FT100K	Stackpole Elect	Digikey
Resistor	7.68k	R3	1	0.10	0.10	RC0805FR-077K68L	Yageo	Digikey
Resistor	2.49k	R4, R9, R14, R19, R24, R29, R34, R39, R44, R49, R54, R59	12	0.02	0.24	RC0805FR-072K49L	Yageo	Digikey
Resistor	22.6k	R5	1	0.10	0.10	RC0805FR-0722K6L	Yageo	Digikey
Resistor	512k	R7, R12	2	0.10	0.20	RC0805FR-07511KL	Yageo	Digikey
Resistor	249k	R10, R15, R20, R25, R30, R35, R40, R45, R50, R55, R60	11	0.02	0.23	RC0805FR-07249KL	Yageo	Digikey
Resistor	27.4	R8, R13, R18, R23, R28, R33, R38, R43, R48, R53	9	0.10	0.90	ERJ-3EKF274RV	Panasonic	Digikey
Resistor	512k	R17, R22, R27, R32, R37, R42, R47, R52, R57	9	0.02	0.19	RC0805FR-07512KL	Yageo	Digikey
Resistor	10k	R61, R64, R66, R69, R71, R72, R98	7	0.10	0.70	RC0805FR-0710KL	Yageo	Digikey
Resistor	100	R62, R63, R65, R67, R68, R70, R73	7		0.00	RC0805FR-07100RL	Yageo	Digikey
Resistor	2.20k	R75	1	0.10	0.10	RC0805FR-072K2L	Yageo	Digikey
Resistor	660	R80, R81	2		0.00	RC0805FR-07660RL	Yageo	Digikey
Op-Amp	MCP642	U1, U2, U3, U4, U5, U6, U7, U8, U9, U10, U11, U12, U14, U15, U16, U17, U18, U19, U20, U21, U22, U23, U24, U25	24	0.40	9.60	MCP6421	Microchip	Microchip
LED Driver	TLC592	U13	1	1.69	1.69	TLC5929	Texas Instrume	Digikey
Microcontroller		U27	1	3.42	3.42	STM8L151C8	ST Micro	Digikey
LDO		U28, U29	2	1.77	3.54	SPX3940	Exar	Digikey
Sensor PCB	Sensor F	Sensor Board	1	15.35	15.35		PCB Express	PCB Express
Breakout PCB	Breakou	Breakout Board	1	8.51	8.51		PCB Express	PCB Express
Flex Cable	18 position		1	4.58	4.58	WM10058-ND	Molex	Digikey
Serial Level Shifter		Max 233	1	6.80	6.80	MAX233EPP+G36	Maxim Integrat	Digikey
Electronics Total					**94.32**			

10.6.2 Mechanical BOM

Component	Quantity	Price	Totals	Part Number	Distributor
PID Controller	1	36.50	36.50	JLD612	LightObject
Insulated Cabinet (*1)	1	55.49	55.49	B000O1TV28	L. A. Liquidators
Hardened Steel Rails (1 foot rod)	1	5.07	5.07	7936k31	McMaster Carr
Brass Bushings	4	0.77	3.08	6338k411	McMaster Carr
Serial Connector	1	1.21	1.21	609-1524-ND	Digikey
Sample Vial Holder	1	7.17	7.17	124A1	SEOH
Delrin Block (anti static)	2	16.06	32.12	8662k72	McMaster Carr
Wire, nuts, bolts, misc Interconnect	1	9.00	9.00		McMaster Carr
Drive Motor	1	11.00	11.00	Nextrox	Hossen
Motor Relay	1	9.45	9.45	SSR-25 DD	Amazon
Heater Housing	1	1.90	1.90	648922	Amazon
Heater Resistors	1	7.22	7.22	pf2472-2rf1	Digikey
Heater Heatsink	1	1.23	1.23	345-1095-ND	Digikey
Heater Relay	1	9.45	9.45	SSR-25 DD	Amazon
Total			**189.89**		

*1 Note Given the availability of numerous sources of incubated cabinets of similar form factor, the design will work on the assumption that the incubated cabinet would be purchased as an OEM component of the system as a whole. The cabinet price represented, is an average of three potential sources of similar characteristics.

10.7 Biographical Statement

Tal Gottesman (born 1977, Syracuse NY) earned his Bachelor of Science degree in Computer Engineering from West Virginia University in 2002. He has worked developing embedded systems in the medical technology industry between 2002 and 2007. Mr. Gottesman has been developing consumer electronics with a focus on motor control, battery management systems and multi chemistry battery chargers since 2007. He received his Master of Science degree in Electrical and Computer Engineering from Johns Hopkins University in 2014 and is currently working as a Senior Project Engineer at Stanley Black and Decker.